# Arsenic in the Pak Pa-Nang River Basin, Thailand

by

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## Abstract

The Pak Pa-Nang River Basin is located in southern Thailand. Its environmental degradation has been arising because its catchment is mineralized with substantial deposits of tin forming part of the SE Asian Tin Belt, with the presence of arsenopyrite gives rise to high arsenic concentrations, mobilized during past mining activity. Suitable techniques have been developed, for the extraction of arsenic species in a variety of environmental and biological samples. Trypsin and cellulase enzymatic extraction procedures were used to extract arsenic species from fish and plant samples, respectively. Arsenic species in sediments were determined following 1 M $H_3PO_4$ extraction in an open focused microwave system.

An anion-exchange HPLC system employing a step elution, with sulphate and phosphate solution as the mobile phase coupled with ICP-MS was used for separation and detection of the important arsenic species, e.g. AsB, DMA, MMA, and inorganic arsenic in fish and plant samples. And species of $As^{III}$, $As^{V}$, MMA and DMA were determined in sediment samples. A nitric acid microwave digestion procedure, followed by carrier gas nitrogen addition ($N_2$)-ICP-MS analysis, to overcome argon chloride ($^{40}Ar^{35}Cl^{+}$) interference, was used to measure total arsenic. Validation for these procedures was carried out using certified reference materials and real samples, mussel, cockle, green seaweed, brown seaweed and sediment collected from the Tamar Estuary, UK.

Fish samples from the Pak Pa-Nang Estuary showed a range for total arsenic concentration, up to 17 $\mu g\ g^{-1}$ dry mass. The highest total arsenic found in plant samples was 189 $\mu g\ g^{-1}$ (dry mass), in the root of rice plants. The major species of arsenic in all fish samples was AsB, together with smaller quantities of DMA and, more importantly, inorganic arsenic. The major species found in plant was MMA, together with inorganic arsenic at various levels, ranging from minor to trace, dependent upon the part of the plant. Total concentrations of arsenic in the sediments covered a range up to 285 $\mu g\ g^{-1}$, and showed a steep decreasing concentration gradient downstream from the upper mined areas to the estuary. $As^{V}$ was the major species found in the sediment samples with smaller quantities of $As^{III}$ and MMA. The presence of the more toxic inorganic forms of arsenic in water, sediments and biota samples has implications for human health, particularly as they are readily 'available'.

Considering the dynamic conditions found in the river basin between the dry and wet (monsoon) season, the supply of these highly toxic arsenic species to humans and environment is likely to continue. This may be for many years, particularly when the levels of arsenic stored in river sediments are considered. Higher rainfall could remobilize arsenic from the various main and intermediate sources and could be carried on SPM, especially on fine particulate matter, to the Pak Pa-Nang Estuary and also the Gulf of Thailand. From this study, the implications of arsenic transport within the water management system for the Pak Pa-Nang River Basin are highlighted.

# Contents

# List of Figures

## Chapter 1



## Chapter 2

## Chapter 3



## Chapter 4

## Chapter 5

# List of Tables

## Chapter 1



## Chapter 2

## Chapter 3



## Chapter 4



## Chapter 5

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## Author 's declaration

At no time during the registration for the degree of Doctor of Philosophy has the author been registered for any other University Award.

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# Chapter 1

# Introduction

## 1.1 Overview of arsenic chemistry

Arsenic is a lustrous grey coloured element, it is classed as a metalloid and it belongs to group 15 in the Periodic Table. The major oxidation states of arsenic are +3 and +5. Elemental arsenic is insoluble in water but soluble in $HNO_3$. Even though arsenic has chemical properties similar to phosphorus, the environmental chemistry of arsenic is different because it can exist in more than one oxidation state under the normal range of environmental conditions, and arsenic can form bonds with sulfur and carbon much more readily than phosphorus[1]. Arsenic is present in many mineral deposits and in particular those containing sulphide minerals, the most common of which is arsenopyrites (FeAsS).

Arsenic has a complex organic chemistry and this is reflected to some extent in its environmental behavior. Complex organic arsenic compounds (Figure 1-1) such as arsenobetaine (AsB), methyl and dimethyl arsenic species, usually reported as monomethylarsonate (MMA) and dimethylarsinate (DMA), tetramethylarsonium salts, arsenocholine (As Chol), dimethyl (ribosyl) arsine oxides, and arsenic-containing lipids have been identified in the marine environment[2-7].

Anthropogenic sources of arsenic arise from agricultural and industrial uses of arsenic given in Table 1-1. Mining, smelting of non-ferrous metals and burning of fossil fuels are the major industrial processes that contribute to anthropogenic arsenic contamination of air, water and soil. Some estimates suggest that 60% of arsenic comes

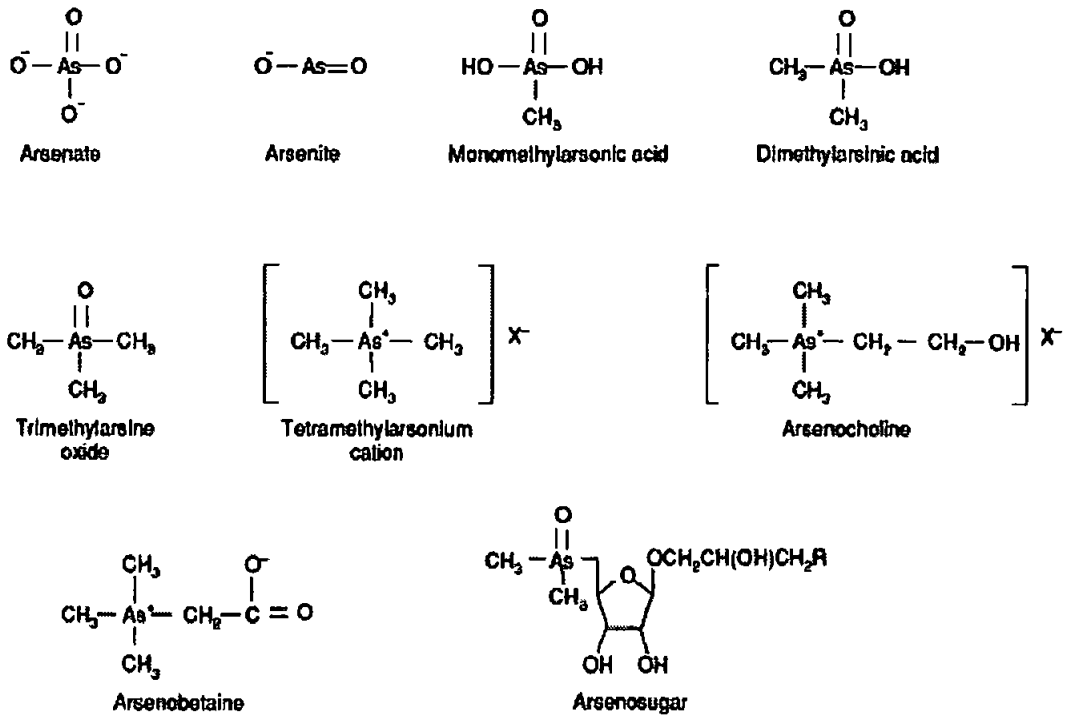


**Figure 1-1 Structures of arsenic species found in marine compartments.**

from natural rather than anthropogenic sources[8]. Exposure to arsenic compounds may occur in a variety of ways through certain industrial effluents, chemical alloys, pesticides, wood preservative agents, combustion of fossil fuels, occupational hazards in mining and dissolution in drinking water[9].

**Table 1-1 Agricultural and industrial uses of arsenic compounds[4].**

| Activity area | Uses |
|---|---|
| Agriculture | Pesticides, insecticides, defoliants, wood preservatives, soil sterilants, e.g. chromated copper arsenate |
| Livestock | Feed additives, disease prevention (swine dysentery), cattle and sheep dips, algaecides, e.g. roxarsone |
| Electronics | Solar cells, optoelectronic devices, semi-conductor applications, light-emitting diodes, e.g. gallium arsenide |
| Industry | Glassware, electro photography, catalysts, pyrotechnics, anti-Fouling paints, dyes, ceramics, e.g. copper arsenate |
| Metallurgy | Alloys, battery plates, e.g. arsenic metal |

### 1.1.1 Arsenic poisoning episodes around the world

Arsenic poisoning has been reported all over the world and examples are given in Table 1-2. Natural arsenic contamination of groundwater is found to be the single most important source of poisoning. The arsenic poisoning episode in Bangladesh has been described as one of the worst cases of arsenic poison in the world. Millions of people in Bangladesh risk exposure to arsenic through consumption of water containing arsenic exceeding the current threshold new value of 10 $\mu g\ l^{-1}$ (set up by the WHO)[6]. More than 3,000 people are suffering from arsenicosis[9].

In southern Thailand, arsenic contamination is generally anthropogenic in nature, due to mining activities. This area has substantial deposits of tin forming part of the SE Asian Tin Belt. The presence of associated arsenopyrite gives rise to high arsenic concentrations, which have been mobilized due to this past activity. Mining has been important in southern Thailand around the Nakorn Si Thammarat province, for over 50 years. More recently these mining sites have been abandoned and become water ponds. Because of the natural rainfall during the monsoon period (September to January) the mine tailings and spoil tips may have been flooded downstream. Contamination also comes from from dissolved arsenic in shallow wells used by the Ronpiboon district villagers (Ronpiboon district has a population of 20,000) for their water supply[9,11]. At many sites, the arsenic content of water exceeds the former regulatory level of 50 $\mu g\ l^{-1}$ (set up by the WHO)[10].

The Pak Pa-Nang river, and its tributaries, are important water sources of the Ronpiboon district and these waters and those in Pak Pa-Nang Estuary, are the focus of this research project. Environmental degradation in the Pak Pa-Nang River Basin has also arisen because the catchment is supplied with the mineralized deposit from the abandoned tin mines. The consequence has been very poor water quality[9,12], detrimental effects on human health[13,14] and on the environment[11,15,16]. Although there have been attempts to reduce the impact from the arsenic contamination[17] the number of cases of cancer arising from arsenic poisoning still appear to be increasing[18].

## 1.2 Toxicity of arsenic

Arsenic first gained notoriety as a poison about 4,000 years ago[6]. However, the toxicity of an arsenic compounds is highly dependent upon its chemical form. The majority of organic arsenic compounds, for example AsB, MMA and DMA are less toxic than inorganic arsenic compounds which differs from other common heavy metals.

**Table 1-2 Arsenic poisoning cases around the world.**

| Location | Population-risk from arsenic poisoning | Arsenic sources | References |
|---|---|---|---|
| Bangladesh | 25 million | National groundwater arsenic contamination | 9 |
| Mexico, region Lagunera | 200,000 | volcanic sediment contamination to drinking water | 19,20 |
| China, Xinjiang , Shanxi, Liaoning, Jilin, Ningxia, Qinghai, and Henan provinces | over 2 million | National groundwater arsenic contamination, range from 0.2–2 mg $l^{-1}$ | 9 |
| India, West Bengal | 6 million | Natural groundwater arsenic contamination | 9 |
| Bulgaria, Srednogorie | 32,000 | Arsenic contamination of soil and river water due to copper smelter concentration in river water range 0.75-1.5 mg $l^{-1}$ | 9 |
| China, Yunan | 100,000 | air-arsenic concentration caused by smelter contaminant | 9 |
| Thailand, Ronpiboon | 20,000 | Arsenic contaminated from Suanchan and Ronna Mountains and arsenic contamination in shallow well water from mining | 9,11 |

This emphasises the need for qualitative and quantitative information on its speciation[21]. In terms of toxicity, the lethal and sub-lethal effects from arsenic species on a variety of taxonomic groups are shown in Table 1-3.

**Table 1-3 Lethal and sub-lethal effects of arsenic compounds on biota.**

| Taxonomic group, Arsenic species | Dose/Media concentration | Effects | Reference |
|---|---|---|---|
| **Rat** | | | |
| $As^{+3}$ | 1.5 mg $kg^{-1}$ bw | $LD_{50}$ | 22 |
| $As^{+5}$ | 5 mg $kg^{-1}$ bw | $LD_{50}$ | 22 |
| MMA | 50 mg $kg^{-1}$ bw | $LD_{50}$ | 22 |
| DMA | 600 mg $kg^{-1}$ bw | $LD_{50}$ | 22 |
| AsB | >10 g $kg^{-1}$ bw | relatively non-toxic | 23 |
| Arsenosugars | - | relatively non-toxic | 24 |
| **Marine invertebrates** | | | |
| Copepod, *Acartia clausi* | | | |
| $As^{+3}$ | 0.51 mg $l^{-1}$ | $LC_{50}$ (96 hr) | 25 |
| Crab, *Cancer magister* | | | |
| $As^{+5}$ | 0.23 mg $l^{-1}$ | $LC_{50}$ (96 hr for zoea) | 25 |
| Oyster, *Crassostrea gigas* | | | |
| $As^{+3}$ | 0.33 mg $l^{-1}$ | $LC_{50}$ (96 hr for embryos) | 25 |
| **Marine vertebrates** | | | |
| Mullet, *Chelon labrosus* | | | |
| $As^{+3}$ | 27.3 mg $l^{-1}$ | $LC_{50}$ (96 hr) | 25 |
| **Marine plants** | | | |
| Phytoplankton | | | |
| $As^{+5}$ | 0.075 mg $l^{-1}$ | Reduced biomass in 4 days | 25 |
| Algae, *Skeletonema costatum* | | | |
| $As^{+5}$ | 0.013 mg $l^{-1}$ | Growth inhibited | 25 |
| Algae, *Thalassiosira aestivalis* | | | |
| $As^{+5}$ | 0.075 mg $l^{-1}$ | Reduced chlorophyll a | 25 |

- bw = body weight.
- $LD_{50}$ represents the median lethal dose, the dose that kills 50% of a population.
- $LC_{50}$ represents the median lethal concentration, the concentration of media that kills 50% of a population.

The $LD_{50}$ value represents the dose that kills 50% of a population. In order to compare the toxicity of various arsenical compounds, $LD_{50}$ values for rats are chosen and shown in Table 1-3. Results of toxicity tests on marine organisms are limited and most concentrate on the more toxic species of arsenic (i.e. inorganic $As^{+3}$ and $As^{+5}$). The chemical species and oxidation states of arsenic are more important as regards to toxicity. The toxicity also depends on other factors, such as the physical state e.g. as a gas or in solution, or powder particle size, the specific surface area, the rate of absorption into cells, the rate of elimination, the nature of chemical substituents in the toxic compound, and the preexisting state of the organism.

### 1.2.1 Metabolism of arsenic and effects on humans

Humans are exposed to different forms of arsenic species in food, water and other environmental media. Each arsenic species has different physicochemical properties and bioavailability. There is a large difference between the various arsenic species compared with other metals and metalloids, which may determine its metabolism. Human intake of arsenic is usually considered in term of (a) oral uptake of arsenic in food, water and beverages or (b) respiratory uptake in dust and fumes, which the absorption rates of arsenic are generally low (<10%)[10]. The bioavailability of inorganic arsenic depends upon the matrix in which it is ingested (i.e. food, water, beverages or particles), the solubility of an arsenical compound itself and the presence of other food constituents and nutrients in the gastrointestinal tract. The fate of ingested arsenic depends on oxidation and reduction reactions between $As^{V}$ and $As^{III}$ in the body plasma and the consecutive methylation reactions in the liver. It is estimated that about 60-70% of the daily-ingested inorganic arsenic is excreted in the urine[26]. For DMA it was found that 3.5% of a single oral dose of 0.1 mg As $kg^{-1}$ was eliminated in urine as trimethylarsine oxide (TMAO) within 3 days[27]. Although arsenic is excreted via other routes than urine, feces and sweat, these routes of excretion are generally small[10]. Since arsenic can accumulate in keratin-containing tissues, skin, hair and nails may also be considered as potentially minor excretory routes. Arsenic can also be excreted in human milk but only at low levels[10,28]. The most commonly employed biomarkers used to identify or quantify arsenic exposure in humans are total arsenic in hair, nails and blood, and the total or speciated metabolites of arsenic in urine. However, arsenic (in the trivalent form) accumulated in keratin-rich tissues such as skin, hair and nails, can only be used as indicators of past arsenic exposure. Normally inorganic arsenic is quickly

cleared from human blood. For this reason blood arsenic is used only as an indicator of recent or relatively high level exposure, for example, in poisoning cases[29] or in cases of chronic stable exposure (i.e. from drinking water). However, since arsenic is rapidly metabolized and excreted in the urine, levels in urine are best suited to indicate recent arsenic exposure. Total arsenic, inorganic arsenic and the sum of arsenic metabolites (inorganic arsenic + MMA + DMA) in urine are therefore all used as biomarkers of recent arsenic exposure[26,27].

- **Dermal effects**

Human chronic exposures to arsenic by either ingestion or inhalation can generate a variety of signs on the skin. These signs of toxicity on the skin are called hyperpigmentation, especially in areas not exposed to the sun, and hyperkeratosis on the palms of hands and soles of feet. The most severe forms of arsenic toxicity cause skin cancer. Skin disorders are developed in people who consume arsenic-containing water in the range 0.01-0.1 mg As $kg^{-1}$ per day[30-34]. Hyper pigmentation may occur, particularly in body areas where the skin tends to be a little darker[35]. Pavittranon et al. (2003)[11] have studied this skin disorder in people living in the arsenic-contaminated area of Ronpiboon District, Thailand. The results show that thousands of people have developed skin lesions in their palms and soles[11]. In some cases, they develop transverse white indentations on fingernails and toenails called the 'Mees' or 'Aldrich-Mees' line. No dermal or other effects result from exposure to chronic doses of 0.003-0.01 mg As $kg^{-1}$ per day[36].

- **Carcinogenic effects**

Patients who received chronic arsenical uptake have a significantly increased incidence of carcinogenic effects of the skin[37]. These arsenical skin cancers normally occur in the presence of dermatologic manifestations of arsenicism. Some have internal neoplasms that are regarded as of arsenicalin origin and much of this type can be found in the population in those parts of Taiwan where it is endemic[38]. In addition, there are reports of elevated cancer risks to a variety of organs (notably lung, skin, bladder, kidney and liver) from other parts of the world including Japan, Bangladesh, West Bengal, India, Chile and Argentina where sections of the population are exposed to arsenic-contaminated drinking water[10, 39].

• **Neurological effects**

Exposure to high concentrations of inorganic arsenic (>1 mg As $kg^{-1}$ per day) regularly causes encephalopathy with symptoms such as headaches, lethargy, mental confusion, hallucinations, seizures and unconsciousness[9]. Intermediate and chronic exposures (0.05-0.5 mg As $kg^{-1}$ per day) are able to cause symmetrical peripheral neuropathy, which is manifest as lack of feeling in hands and feet. The patients will possibly develop a painful 'pins and needles' sensation, asymmetric bilateral phrenic nerve and peripheral neuropathy of both sensory and motor neurons causing numbness, loss of reflexes, and muscle weakness[40].

## 1.3 Guideline recommended limiting values of arsenic for safety

In 1993 the World Health Organization (WHO) made a provisional guideline recommendation of 10 μg $l^{-1}$ for arsenic (total) in drinking water[6]. Before 1993, the WHO drinking water guideline value for arsenic (total) was 50 μg $l^{-1}$, which corresponds to a daily intake of 100-200 μg[2]. In the WHO air quality guidelines for Europe it was concluded that, because inorganic arsenic is carcinogenic and there is no safe threshold, no safe level for arsenic can be recommended[3]. The WHO has estimated the human lifetime risk of getting lung cancer from inorganic arsenic in air as 0.0015 for 1 μg As $m^{-3}$ exposure. WHO has recommended that the daily oral intake of inorganic arsenic should not exceed 2 μg arsenic $kg^{-1}$ body weight. The Joint FAO/WHO Expert Committee on Food Additives assigned a provisional tolerable weekly intake (PTWI) of 15 μg inorganic arsenic $kg^{-1}$ body weight, but stressed that there is a narrow margin between the PTWI and those intakes reported in epidemiological studies to have toxic effects[3]. There are indications that arsenic may be essential, at least for some animals. If it is essential also for humans, the requirement for arsenic is probably close to 20 μg $day^{-1}$ (WHO 1996)[41]. The PTWI value (15 μg) corresponds to a maximum daily intake of 150 μg for a human weighing 70 kg. For other biota, it has been estimated that rice-yield decrease by 10 % when its soil subject to level of 25 mg arsenic $kg^{-1}$ [42]. Eisler (1994)[25] has put together a list of criteria for arsenic that has been proposed by authorities and experts from different countries in order to protect natural resources and human health.

## 1.4 Distribution of arsenic in the environment

### 1.4.1 Arsenic in rocks, soils, ground water and terrestrial plants

Concentrations of arsenic found in soils are higher than those found in rocks[43]. Uncontaminated soils usually contain 1-40 $\mu g\ g^{-1}$ of arsenic, with lowest concentrations in sandy soils and those derived from granites, whereas larger concentrations are found in alluvial and organic soils[44]. The levels of arsenic in soils from various countries are in normal ranges, except in some contaminated areas such as West Bengal, India (Table 1-4).

**Table 1-4 Concentrations of arsenic in soils and sediments[9].**

| Country | Types of soil/sediment | Concentration range ($\mu g\ g^{-1}$) | Mean ($\mu g\ g^{-1}$) |
|---|---|---|---|
| India, West Bengal | Sediments | 10–196 | - |
| Japan | Paddy | 1.2–38.2 | 9 |
| Argentina | All types | 0.8-22 | 5 |
| France | All types | 0.1-5 | 2 |
| Mexico | All types | 2-40 | 14 |
| South Africa | Not classified | 3.2-3.7 | 3 |

Parent rock and human activities are important factors influencing the composition of arsenic in soils. Factors such as climate, ratio of organic and inorganic components of the soils and redox potential status also regulate the level of arsenic in soils. Arsenic from rock weathering occurs mainly as inorganic species then can bind to organic materials in soils. Under oxidizing conditions, in aerobic environments, $As^{V}$ is the dominant species and is strongly sorbed onto clays, iron and manganese oxides/hydroxides and organic matter. Arsenic easily precipitates as ferric arsenate in soil rich in iron. Under reducing conditions $As^{III}$ is the dominant arsenic species. Inorganic arsenic species can be methylated by microorganisms, producing under oxidizing conditions, MMA, DMA and TMAO. The species of arsenic present in soils depend on a variety of factors such as, type and amount of sorbing components in the soil, pH and the redox potential. Fe and Al arsenate ($AlAsO_4$, $FeAsO_4$) are the dominant

phases in acid soils and are less soluble than calcium arsenate ($Ca_3AsO_4$), which is the main chemical form in any alkaline and calcareous soil. The absorption of arsenate in soils is related to soil pH and redox potential (Eh) (Figure 1-2). It also varies with soil type under the same pH conditions. Biological activities can form methylarsenic (addition of $CH_3$ to arsenic through biological activity) in soil-water, sediment water interfaces through the activity of bacteria, such as *Escherichia coli*, *Flavobacterium* sp, *Methanobacterium* sp and fungi, such as *Aspergillus glaucus* and *Candida humicol*a. In biomethylation processes, $As^{III}$ is oxidized to $As^{V}$ and stable methylated arsenic species are formed[9,46].

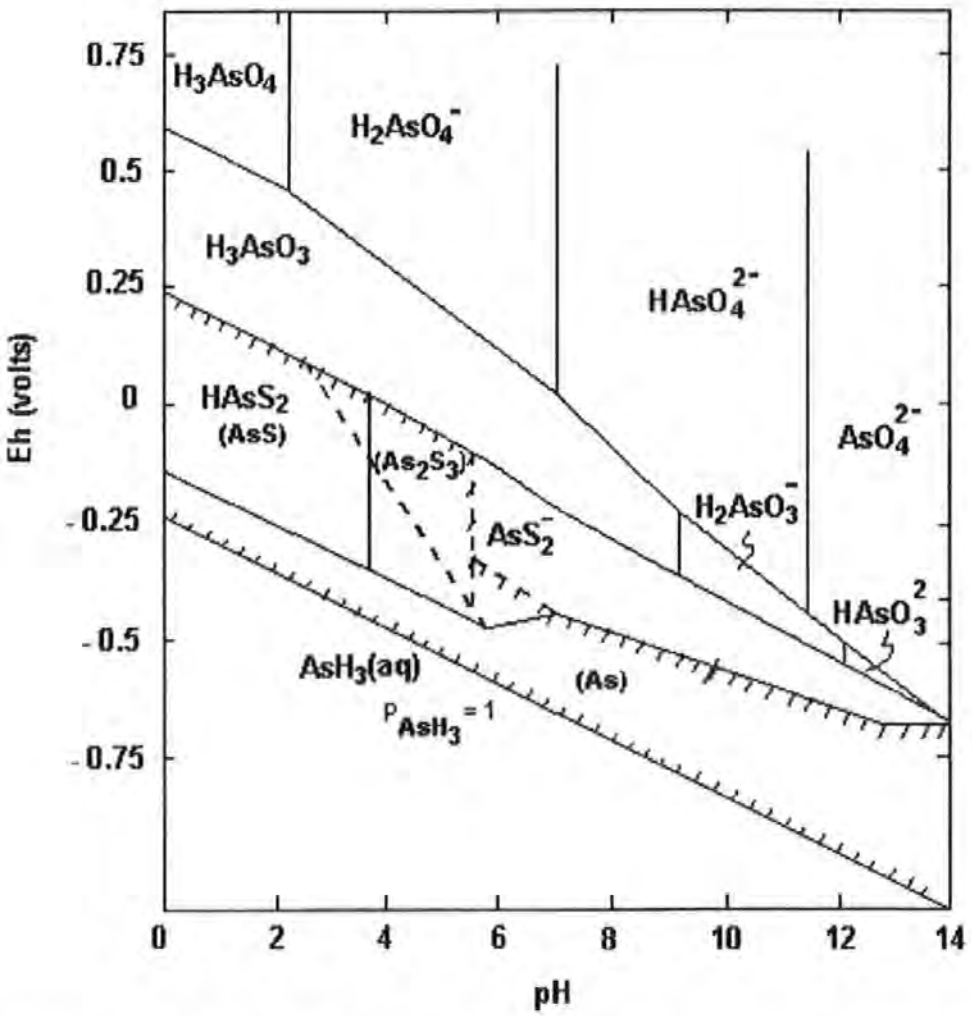


**Figure 1-2 Eh-pH diagram for the arsenic water-sediment interface at 25°C and one atmosphere pressure (Source: Ferguson, J.F. and Gavis, J. 1972)[45].**

Arsenic levels in uncontaminated groundwater usually range from 1-2 μg $l^{-1}$. The predominant arsenic species in ground water is arsenate with miner species being

arsenite. Arsenic from weathered rocks and soils with high concentration arsenic may leach soluble forms into surface water or groundwater. In some contaminated areas the concentrations of arsenic in ground water can be found as high as hundreds or thousands $\mu g\ l^{-1}$ and some examples are summarized in Table 1-5[47,48].

In a mining area of Western USA, elevated arsenic concentrations in ground water were up to 48 $mg\ l^{-1}$ [51]. Elevated concentrations of arsenic in ground water close to a mining area were also found in the Nakorn Si Thammarat province of Thailand where the concentrations ranged from 1.25 to 5114 $\mu g\ l^{-1}$ [13]. Other areas of the world, such as in India and Bangladesh[49], have high arsenic concentrations in ground water arising from contact with natural arsenic-rich sediments.

**Table 1-5 Concentrations of arsenic found in contaminated groundwater.**

| Location | Sampling period | Arsenic source | Concentration ($\mu g\ l^{-1}$) | Reference |
|---|---|---|---|---|
| Thailand, Nakhon Si Thammarat Province, | 1994 | Shallow ground-water; mining activity | 1.25-5114 | 13 |
| Bangladesh | 1996-1997 | As-rich sediment | 10->1000 | 49,50 |
| West Bengal, India | ns | As-rich sediment | 193-737 | 49 |
| Western USA | ns | Mining activity | Up to 48,000 | 51 |

ns = not stated

Arsenic is metabolised from inorganic to organic forms by a wide range of terrestrial plants. Metabolism typically occurs through biomethylation to give MMA, DMA, tetramethylarsonium ions (TeMA) and trimethylarsine oxide (TMAO)[52] (Figure 1-3). Further metabolism to form arsenocholine, arsenobetaine and arsenosugars occurs in a number of organisms, and these compounds have been measured in some terrestrial plants. Some factors such as arsenic species present in the soil; the ability of the compounds to enter the plant, actively or passively; the ability of the plant to synthesise arsenic species; and the presence of arsenic species adsorbed to the outside surface of the plant roots can also influence the arsenic species present in a plant[53].

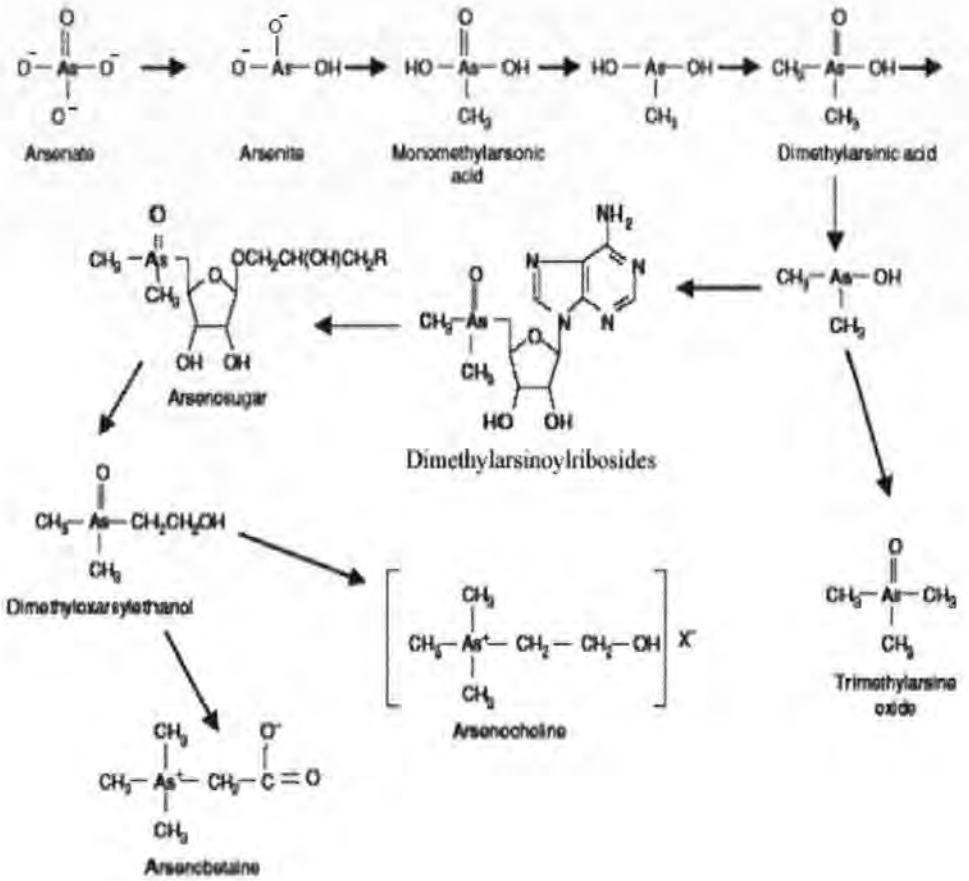


**Figure 1-3 Potential pathways for the reduction and methylation of arsenic by terrestrial plants (Source: Meharg, A.A. and Hartley-Whitaker, A. 2002)[53].**

### 1.4.2 Arsenic in river waters

Examples of the concentrations of total arsenic in surface freshwaters are summarized in Table 1-6. The levels of uncontaminated rivers and lakes are generally found to be below 10 μg $l^{-1}$; although individual samples may range up to 1 mg $l^{-1}$ 47[47,51,54]. The relatively low background concentrations of arsenic are found in the Bowron Lake, Canada and in the River Amazon, where dissolved arsenic concentrations are decreased by scavenging by iron-rich particles. In contrast, the Yangtze River, China with concentrations range from 0.1-28 μg $l^{-1}$, is impacted by mining and geothermal activity and pesticide production. Mean total arsenic concentrations of 2,000 μg $l^{-1}$ have been recorded near a pesticide plant, with MMA being the predominant arsenic species[55]. In Thailand, where tin mining is the source of contamination, the concentration of arsenic in contaminated surface water ranges from 4.8 to 583 μg $l^{-1}$. Geothermal and volcanic

sources of arsenic have also been found in the Madison River, USA and Waikato River, New Zealand, where the concentrations range from 28 to 51 $\mu g\ l^{-1}$.

**Table 1-6 Concentrations of dissolved arsenic in surface freshwaters.**

| Location | Sampling period | As source | Concentration ($\mu g\ l^{-1}$) | Reference |
|---|---|---|---|---|
| Maurice river, NJ, USA | 1982-1983 | upstream of pesticide plant | 1.05-4.4 | 55 |
| | 1982-1983 | 0.6 km downstream | 1,320-4,160 | 55 |
| | 1982-1983 | 4.2 km downstream | 118-578 | 55 |
| Yangtze river (source area), China | Not stated | no mining activity | 0.1-28.3 | 56 |
| Bowron lake, British Columbia, Canada | 1992 | Reference lake; no mining activity | <0.2-0.42 | 54 |
| Nakhon Si Thammarat, Thailand | 1994 | mining activity | 4.8-583 | 13 |
| Madison river, Montana, USA | Not stated | geothermal | 51 | 57 |
| Waikato river, New Zealand | 1993-1994 | volcanic source | 28.4-35.8 | 58 |
| River Amazon, Brazil | 1996 | Weathering | 0.21 | 59 |

### 1.4.3 Arsenic in estuarine and coastal waters

The concentrations of arsenic found in estuarine waters and sediment pore waters are summarized in Table 1-7. Dissolved inorganic arsenic has been reported as major arsenic species in estuarine waters. Interactions between water, biota, suspended particles and sediments can convert arsenic species in water, with the dominant organic compounds detected in estuarine water being MMA and DMA. However, MMA and DMA appear only to be present in the period during spring and summer[60,61]. During winter, when there is low water temperature and low chlorophyll concentrations only dissolved inorganic arsenic is detected. This indicates that MMA and DMA generated

from the decomposition of phytoplankton, may be demethylated by bacteria which produce the inorganic forms during the autumn and winter[67, 68].

**Table 1-7 Examples of concentrations of dissolved arsenic in estuarine waters.**

| Location | Sampling Period | Arsenic concentration (μg As $l^{-1}$) | | | Ref. |
|---|---|---|---|---|---|
| | | Total Inorganic | DMA | MMA | |
| **Dissolved arsenic** | | | | | |
| Itchen Estuary, UK | 1983 (Winter) | nd | nd | nd | 61 |
| | 1984 (Summer) | 0.04-0.18 | 0.05-0.24 | 0.04-0.06 | |
| St Lawrence Estuary, Canada | 1986 | 0.08-1.4 | nd | nd | 62 |
| Humber Estuary, UK | 1988 (Winter) | 2.6±0.3 | nd | nd | 60 |
| | 1990 (Spring) | 1.3±0.2 | nd | 0.05±0.02 | |
| | 1990 (Summer) | 1.2±0.2 | 0.05±0.02 | 0.21±0.05 | |
| Thames Estuary, UK | 1989 (Winter) | 3.3±0.9 | nd | nd | 63 |
| | 1990 (Summer) | 2.1±1.1 | 0.07±0.03 | 0.25±0.23 | |
| Carnon Estuary, UK | 1992 | <0.4-158 | <0.15-0.5 | <0.2-2.1 | 64 |
| Amazon Plume, Brazil | 1996 | 0.08 | 0.01±0.004 | 0.02 | 59 |
| Tinto Estuary, Spain | 1997 (June) | 200 | | | 64 |
| | 1998 (April) | 132 | | | |
| | | 0.2 ($As^{III}$) | | | |
| **Sediment porewaters** | | | | | |
| Tamar Estuary, UK | 1986 (Annual Range) | 5-62 | 0.04-0.53 | 0.17-0.49 | 66 |
| Thames Estuary, UK | 1989 | 0.4-17 | nd | nd | 63 |
| Humber Estuary, UK | 1990 | 0.5-29 | 0.06-72 | 0.14-0.69 | 60 |

nd=under detection limit.

Levels of arsenic in seawater are summarized in Table 1-8. Concentrations of arsenic in open ocean seawater are typically 1-2 μg $l^{-1}$. The dissolved forms of arsenic in seawater include arsenate, arsenite, MMA and DMA, with adsorption on to particulate matter being the physical process most likely to limit dissolved arsenic concentrations[76].

$As^{III}$ and $As^{V}$ have been revealed as the major arsenic species in seawater since they were carried out by Atkins and Wilson in 1926. Since then these species have been confirmed in subsequent studies[77,78]. Thermodynamic calculations indicate that arsenic in oxygenated seawater should exist almost entirely as arsenate; biological reduction, however, can produce appreciable levels of arsenite[79]. Methyl and dimethyl arsenic species are also detected in seawater. These species result from the uptake and subsequent biotransformation of arsenate by phytoplankton[61,66,80-85]. Apart from $As^{III}$, $As^{V}$, MMA and DMA, other arsenic species have been reported in seawater[80,81,84].

**Table 1-8 Background concentrations of arsenic in seawater.**

| Location | Sampling period | Sampling details and/or species | Concentration ($\mu g\ As\ l^{-1}$) | Reference |
|---|---|---|---|---|
| Gulf of Mexico | ns | 0.2 μm filtered | 0.04 | 69 |
| Coastal waters, Southampton, UK | 1988 | Whatman GF/G filtered | 0.01-0.19 ($As^{III}$)<br>0.04-0.1 (MMA)<br>nd-0.27 (DMA) | 70 |
| Coastal waters, South Australia | ns | dissolved; particulate As below limit of detection (0.6 ng $l^{-1}$) | 1.1-1.6 | 71 |
| Coastal waters, south-east Spain | ns | below surface | 0.45-3.7 | 72 |
| Baltic Sea | 1982-1983 | 0.45 μm filtered | 0.45-1.1 | 73 |
| Coastal waters, Malaysia | ns | 0.45 μm filtered; 66% arsenate; 33% arsenite | 0.65-1.8 | 74 |
| Dover Strait, France | 1990 | GFF filtered Values for ($As^{III}$ + $As^{V}$) MMA+DMA <1% of total in winter and <10% in summer | 1.38±0.12 (winter)<br>1.26±0.03 (summer) | 75 |
| N. Atlantic Ocean Surface Water | 1993 | 0.4 μm filtered; | 0.03±0.02 ($As^{III}$)<br>1.1±0.08 ($As^{V}$)<br>0.01±0.01 (DMA) | 59 |
| N. Atlantic Deep Water | 1993 | 0.4 μm filtered | 1.3±0.2 ($As^{III}$ + $As^{V}$) | 59 |

ns=not stated; nd=under detection limit.

### 1.4.4 Arsenic in aquatic sediments

Sediments in aquatic systems often have higher arsenic concentrations than those of the water[51]. The typical background concentrations of 1-50 μg $g^{-1}$ have been reported[86]. In contaminated areas, arsenic concentrations in aquatic sediments can be raised up to hundreds or thousands μg $g^{-1}$. Examples include various case studies e.g. mean total arsenic concentrations of 500 μg $g^{-1}$ (dry weight) were measured in sediment near a pesticide plant[55]. Bright et al. (1996) found total arsenic concentrations ranging from 1043 to 3090 μg $g^{-1}$ in the top 10 cm of sediment from subarctic lakes contaminated by gold-mining activity[87]. Chunguo and Zihui (1988) studied arsenic accumulation in sediment of the Xiangjiang river (China), which receives inputs from a variety of industrial plants. Total arsenic concentrations upstream of industrial inputs were 13.2 μg $g^{-1}$ during the rainy season and 81.4 μg $g^{-1}$ during the dry season. Near to industrial discharges, maximum total arsenic concentrations exceeded 1,000 μg $g^{-1}$ during the dry season (approximately 70% as iron or aluminum arsenate) but rarely reached 100 μg $g^{-1}$ during the rainy season[88]. Sediment bound arsenic is generally regarded as less available to biota even though its concentrations can be high. The interstitial waters or porewaters of sediments contain bioavailable arsenic, similar to those reported for seawater: inorganic arsenic predominates and MMA and DMA can occur at low but significant levels (e.g.1-4% total arsenic)[66,89,90]. Ebdon et al. (1987) reported that methylated arsenic species represented 1-4% of the total arsenic in sediment porewater from the Tamar estuary, south-west England (United Kingdom)[66]. Similar findings were reported by de Bettencourt (1988) for the Tagus Estuary (Portugal)[90]. In addition, trimethylated arsenic species, presumably TMAO, has been found with MMA and DMA in pore water samples[89]. The concentrations of total dissolved arsenic in porewaters are generally higher than those in seawater[91]. Arsenic species found in different marine compartments are shown in Table 1-9.

### 1.4.5 Arsenic in aquatic biota

Arsenic is a cumulative substance in living tissue, i.e. once ingested by any organism it is passed out of the organism slowly. The amount of arsenic in a plant depends almost solely on the amount of arsenic to which it is exposed. There are a substantial number of publications on the levels of arsenic in marine biota. The following examples in Table 1-10 have been chosen to provide an overview.

**Table 1-9 Arsenic species in different marine compartments[92].**

| Matrix | Arsenic species | | | |
|---|---|---|---|---|
| | Major | Minor | Trace | Not detected |
| Seawater | $As^{V}$, $As^{III}$ | MMA, DMA | - | AsB, TMA, As Chol, arsenosugars |
| Sediment and porewater | $As^{V}$, $As^{III}$ | MMA, DMA | TMAO | AsB, TMA, As Chol, arsenosugars |
| Marine flora | Arsenosugars | $As^{V}$ | MMA, DMA | AsB, TeMA, As Chol |
| Marine fauna | AsB | TMA, arsenosugars | $As^{V}$, $As^{III}$, TMAO, As Chol, DMA | - |

Arsenic is cumulative in animal tissue, producing a wide range of concentration due to the variable levels of arsenic ingested and exposed in different areas. Among marine animals, such as molluscs and crustaceans, the level of total arsenic is from less than 0.2 to 16 $\mu g\ g^{-1}$(dry weight basis).The concentration of arsenic in algae varies from less than 0.05 to about 80 $\mu g\ g^{-1}$ dry weight; the high concentrations are found in brown algae. Algae contain a variety of arsenic species and are regarded as the greatest number of arsenic species container among marine samples. Most of the arsenic in algae is bound in molecules, referred to as arsenosugars. Most of the algal arsenic is present as one or more of the four major compounds shown in Figure 1-4[93-99]. Although most works in the past have been carried out on macroalgae, a study of the unicellular alga, *Chaetoceros concavicornis* also shows arsenosugar compounds[100], suggesting that these compounds are likely to be general algal metabolites. The distribution of the arsenic compounds among algae may have taxonomic significance. For example, in brown algae the major compounds are the arsenosugars 1 and 3 whereas arsenosugars 2 and 4 predominate in red and green algae[91]. Three species so far examined in the order Fucales (*Hizikia fusiforme* and *Sargassum* spp) contain arsenosugar 3 as the major arsenic compound, whereas the three Laminariales species (*Ecklonia radiata*, *Laminaria japonica*, and *Undaria pinnatifida*) contain arsenosugar 1 as the major compound. Other arsenic species commonly found in algae are arsenate, MMA and DMA. These species are generally minor constituents although some high concentrations of arsenate were found in two species of brown algae[101-103].

**Table 1-10 Concentrations of 'total' arsenic in various marine aquatic biota [25].**

| Organisms, species | Concentration μg As $g^{-1}$ |
|---|---|
| **Algae** | |
| Green | 0.05-5 dw |
| Brown | 2-58 dw |
| Brown alga, *Fucus vesiculosus* | 35-80 dw |
| **Molluscs** | |
| Mussel, *Mytilus edulis* | 1.6-16 dw |
| Oysters, *Crassostrea spp.* | 1.3-10 dw |
| | 0.3-3.4 fw |
| **Crustaceans** | |
| Crabs and shrimps | <0.2 dw |
| American lobster, *Homarus americanus* | 3.8-7.6 dw |
| Brown shrimp, *Penaeus aztecus* | 3.1-5.2 fw |
| White shrimp, *Penaeus setiferus* | 1.7-7.7 fw |
| **Fish** | |
| Finfish, Netherlands | 2.8-10.9 fw |
| Finfish, North America | 0.6-30 fw |
| Finfish, Hong Kong | Max 21.1 fw |
| Black sea bass, *Centropristis striata* | 6.4 dw |
| Mullet, *Mugil cephalus* | Max 1.3 fw |

dw=dry weight basis; fw=wet weight basis

The major arsenic compound in marine animals is arsenobetaine. Since its first isolation and identification from the western rock lobster was established in 1977[104], this stable quaternary arsenic compound has been shown to be present in virtually all marine invertebrates and fish, and in most cases it is by far the predominant arsenic species[105,106]. The tetramethylarsonium ion is also commonly found in marine animals[107-110], particularly in bivalve molluscs where it can be the major form[110]. Arsenosugars are found in herbivorous marine animals where the source is almost certainly the marine algae on which the animals feed. With few exceptions, however, arsenosugars are a minor arsenic species in herbivorous marine animals, and they are generally absent at higher trophic levels[82].

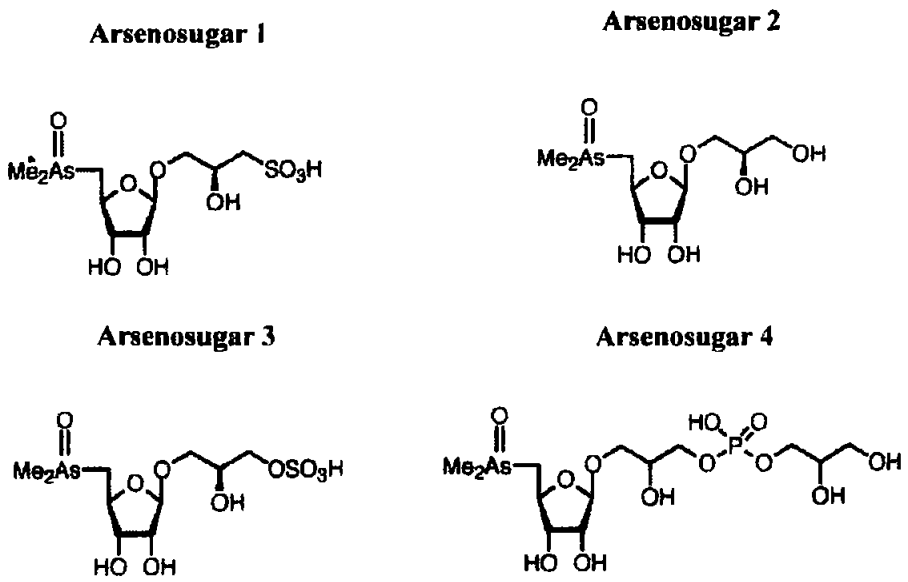


**Figure 1-4 Structures of arsenic species in marine algae.**

## 1.5 Arsenic cycling in aquatic environments

A schematic diagram of arsenic cycling in an aquatic environment[111] is shown in Figure 1-5. Dissolved arsenic can occur in natural waters in both inorganic and organic forms. Dissolved inorganic forms included formal oxidation states $As^{V}$, arsenate and $As^{III}$, arsenite and once utilized by organisms predominant in oxic-photic zone, arsenic is detoxified by conversion to organoarsenic species[112]. The organoarsenicals typically produced in algae biosynthesis are MMA, DMA, TMAO and TMA. Because different organisms produce different organic species[52], the biota present may determine the presence of specific organoarsenic species. In the oxic-photic zone, the dominant arsenic species are inorganic arsenate and organic DMA. The DMA domination could result from direct extraction from algae, microbes or degradation of excreted arsenicals[111,113,114]. In sub-oxic zone, dissolution of Mn and Fe results in a release of adsorbed or coprecipitated arsenic in the hypolimnion. Under sulphase reducing conditions, arsenate can be reduced to arsenite and evidence exists for the removal of an arsenic-enriched mineral phase in this zone.

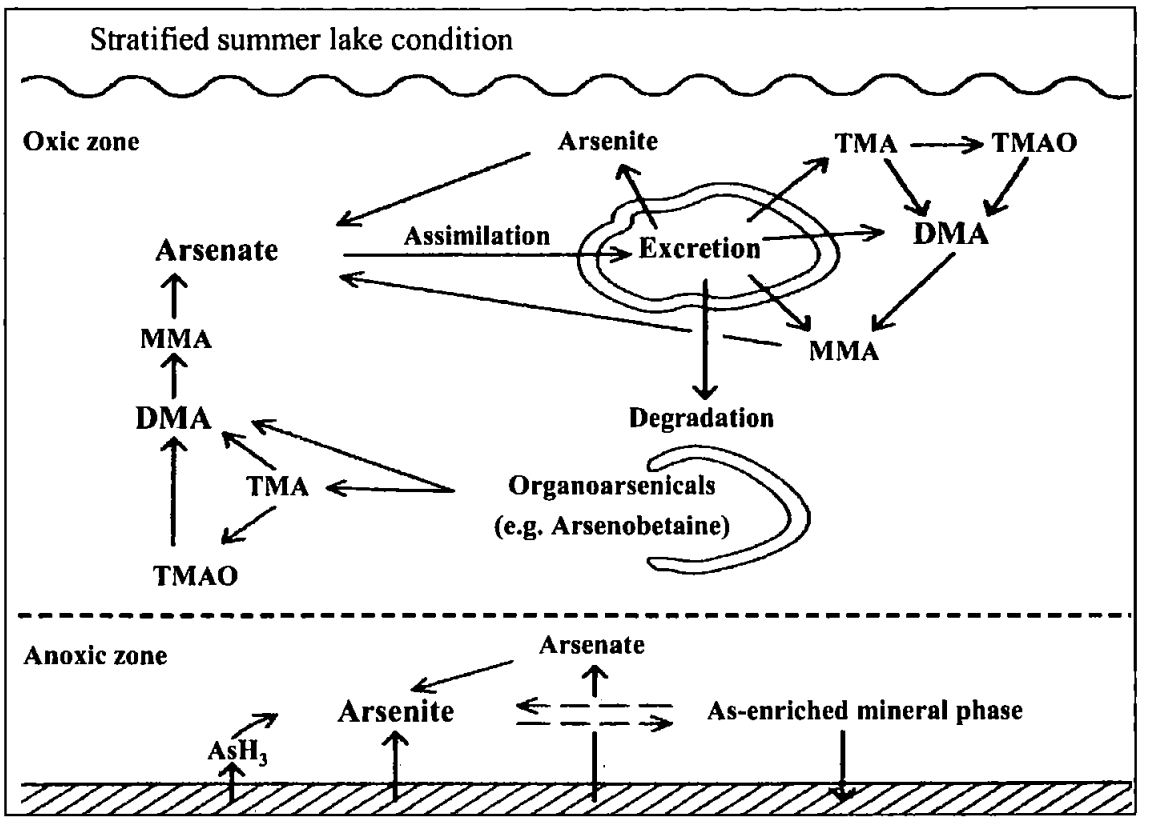


**Figure 1-5 Schematic diagram of the arsenic cycle in the aquatic environment (Source: Anderson and Bruland, 1991)[111].**

## 1.6 Methods for the speciation of arsenic

Since the early 20th century, the term "trace elements" recognized the fact that many elements occurred in such naturally low concentrations that their occurrence could only just be detected by instruments in that period. More recently, efforts were focused on 'total' trace element concentrations and improving the sensitivity of the detectors. In the early 1960s, questions were raised regarding the chemical form of the trace elements and analytical methodologies were developed subsequently. These developments have been growing rapidly and element analysis today appears almost focused on trace element species[21]. The performance of instrumentation has been fundamental to the development of elemental speciation studies. There has been a trend towards lower detection limits in optical atomic spectrometry and mass spectrometry. This has allowed the barrier between 'total' element and elemental species to be passed. The background concentration of elemental species of anthropogenic origin was originally zero. Today they are present, because they have been and continue to be globally distributed in a manner that affects the natural life cycle. However, because some remain below

detection in living systems this does not mean that their presence is non-toxic. At the same time it is also possible that a certain background level of these anthropogenic substances can be tolerated without any adverse effect. In order to assess the impact of low background levels of element species, the separation and detection techniques that surpass the performance of the existing speciation methodology have yet to be developed [21].

### 1.6.1 The extraction of arsenic from samples

For the speciation of arsenic, particularly from solid samples, a solvent extraction procedure is often required before analysis. The most important property of an extractant in the speciation study is to extract species from the samples without modifying them. In addition, the efficiency of the extractant to extract the species from the samples is also important and must be high.

Enzymatic extraction using trypsin (a mammalian digestive enzyme used to attack proteinaceous tissues) provides a high extraction efficiency and high percentage of recovery whilst maintaining the integrity of the arsenic species in the biota sample. Previously, this extraction technique has been used successfully to extract arsenic species from fish samples[115] and baby food samples[116]. Phosphoric acid has been used successfully in the extraction for arsenic species in soils and sediments with high efficiency, recoveries and without modifying them[117,118].

### 1.6.2 Atomic absorption spectrometry (AAS)

The common flame atomic absorption spectrometry method has been used for arsenic determination in many laboratories. However, it is less sensitive than electrothermal or graphite furnace AAS (GFAAS) and hydride generation AAS (HGAAS). Its detection limit is usually in the range of mg As $l^{-1}$, and therefore it has limited application, being especially suited to environmental samples which contain higher levels of arsenic. On the other hand, GFAAS is known as one of the more sensitive atomic spectroscopic methods which can achieve detection limits in the range of nanogram of arsenic[119].

### 1.6.3 The coupling of hydride generation and AAS

Hydride generation coupled with atomic spectroscopy is a popular method for determining hydride reducible arsenic compounds, such as $As^{III}$, $As^{V}$, MMA and DMA. It is noted that compounds such as arsenobetaine and arsenocholine which do not form a volatile arsine are not detected directly by this technique[78-79]. Using HGAAS to determine total arsenic, arsenite and arsenate in foods after a chloroform extraction procedure has been reported, with a recovery of more than 80%[120]. Similar methods have been developed for arsenic speciation[121]. A detection limit of 0.017 μg As $g^{-1}$ was reported when using of HGAAS in the determination of arsenic in seafood products[122]. HGAAS has also been widely employed for the determination of arsenic in water[49].

A schematic diagram of the HGAAS analytical system is shown in Figure 1-6[66]. By forming arsine gas, the analyte is easily and efficiently separated from its sample matrix and transported to the detection system. This technique can reach a lower detection limit using a cryogenic, pre-concentration technique before detection. The hydride generation for arsenic detection can be divided into two stages. The first stage is the reduction of the analyte from an acidified aqueous sample and the evolution of the arsine. The second stage involves the transport of arsine from the generating solution to the detection system; this may also incorporate a pre-concentration and species-separation system.

### 1.6.4 Chromatographic methods for the separation of species

In 1930's the first chromatographic separation was successfully carried out. Since then, few consistent chromatographic methods were commercially available to the laboratory scientist. During 1970's, most chemical separations were carried out using a variety of techniques including open-column chromatography, paper chromatography, and thin layer chromatography. However, these chromatographic techniques were inadequate for quantification of compounds and resolution between similar compounds. High pressure liquid chromatography (HPLC) was developed in the mid-1970's and rapidly improved with the development of column packing materials and the additional convenience of on-line detectors. By the 1980's HPLC was generally used for the separation of chemical compounds. New techniques improved separation, identification, purification and quantification far above the previous techniques[123].

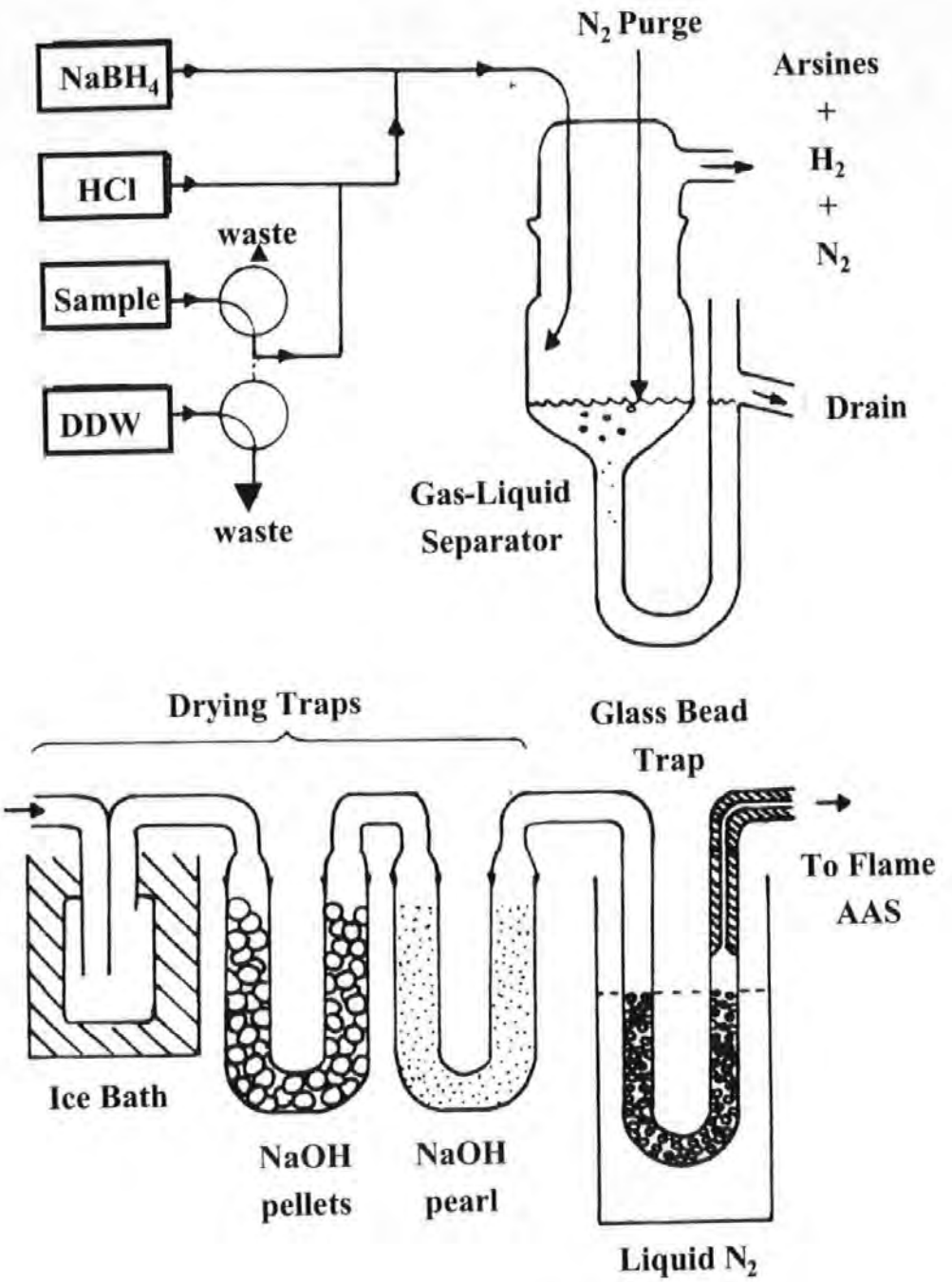


Figure 1-6 Schematic diagram of a hydride generation and pre-concentration method using a cryogenic cold trap (Source: Ebdon et al. 1987)[66].

In an HPLC system, the chromatographic column packing material is an important part of the separation process. Ion-Exchange chromatography is one of the most efficient, which operates on the basis of selective exchange of ions in the sample solution with counter-ions in the stationary phase[124]. It is performed within columns containing charge-bearing functional groups attached to a polymer matrix packing material. The functional ions are permanently bonded to the column and each has a counter-ion attached. The sample is retained by replacing the counter-ion of the stationary phase with its own ions. The sample is eluted from the column by changing the properties of the mobile phase so that the mobile phase will now displace the sample ions from the stationary phase, (ie. changing the pH, ionic strength). Ion-Exchange can be influenced by the choice of the counter ion, since single charged ions are bound less strong than multiply charged ions. The retention time in anion-exchange is increased if a counter ion is exchanged with another. Other effects on ion-exchange chromatography include column temperatures. The higher temperature then the faster the rate of diffusion, which gives the rise to better peak shapes and shorter elution times[124].

### 1.6.5 Inductively coupled plasma-mass spectrometry (ICP-MS)

The ICP-MS is extensively used as a detector for either multi-element or single element analysis and it has the advantage that it can be coupled with various separation techniques. The majority of elements in the Periodic Table, in the mass range 6 to 238 m/z, including As-75, can be quantified, if abundances and interferences permit. The high temperature of the plasma ensures almost complete decomposition of the sample into the constituent atoms and the ionization conditions[125]. A schematic diagram of an ICP-MS instrument is shown in Figure 1-7.

Liquid samples introduced into an ICP must usually contain less than 0.1% of dissolved salts to prevent salt build-up on the sampling cone. Typical sample introduction systems comprise a nebulizer and a spray chamber where the sample is converted to an aerosol. The small droplets pass through the spray chamber to the plasma by the nebulizer gas flow where it is desolvated, decomposed and finally ionized by the high temperature of the plasma. The analyte ions are subsequently introduced in to the MS detector by a chamber held at consecutively lower pressures. Mass analysis is a simple method of separating ions of different mass-to-charge ratio (m/z).

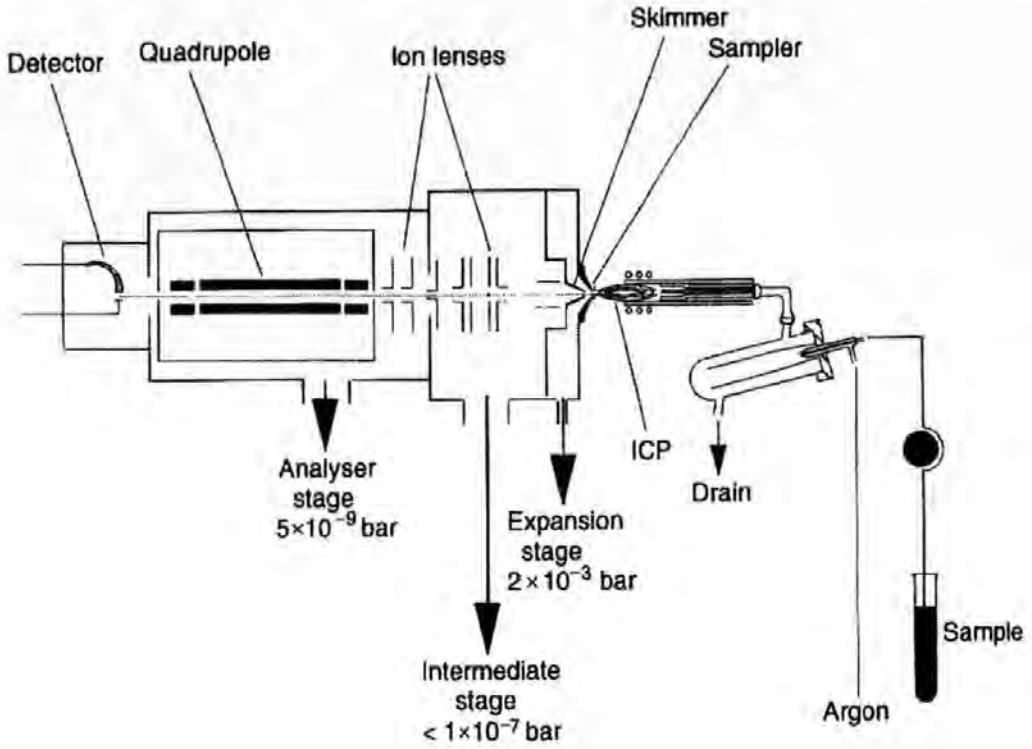


**Figure 1-7 Schematic diagram of an ICP-MS instrument (Source: Hill, S.J. 1999)[125].**

The ions are focused by ion lenses then separated accordingly to their mass-charge ratio either by using a quadrupole (low resolution MS) or magnetic sector (high resolution MS) analyser and finally detected by an electron multiplier or, for high resolution determinations, a Faraday cup detector (depending on analyte concentrations)[125].

The major advantages that the ICP-MS has over the other techniques are its low detection limits, 1-10 pg $ml^{-1}$ (5 pg $ml^{-1}$ for As) range for quadrupole instruments, large linear dynamic range and rapid multi-element capability. However, spectroscopic and non-spectroscopic interferences can limit the utilization of this technique. Spectroscopic interferences include isobaric overlap of element isotopes and polyatomic ions which can be formed from plasma gases, water and from compounds present in the sample matrix. Such interferences can cause an erroneously large signal at the m/z of interest. A major polyatomic interference for arsenic [$As^+$ (monoisotopic m/z 75)] is $^{40}Ar^{35}Cl^+$. Despite the high temperature in the ICP, molecular ions, originating from the plasma gas (Ar), entrained air, the solvent or the matrix, occur in ICP-MS and their signal may complicate the mass spectrum and analyte quantification to a large extent, especially for complex matrices and particularly in the lower mass range (<80 amu). Problems can be

avoided by appropriate selection of the nuclide monitored, although selecting an isotope with a lower abundance obviously leads to deterioration in sensitivity. This is particularly seen in speciation work, where total element concentrations that are already low are further distributed over different species. Some spectroscopic interferences can be overcome by employing a mixed gas plasma. One example of this is the reduction of $^{40}Ar^{35}Cl^{+}$ species (a poly atomic interference on mono atomic $^{75}As^{+}$ in high chloride content samples) by the addition of $N_2$ gas to the nebulizer flow, which has been used successfully by a number of workers[126].

This advantage may be explained by the competitive formation for $ArN^{+}$. Increasing the nebulizer flow rate can also reduce certain polyatomic interferences as there is less time spent in the plasma and therefore less time for the formation of these interferences. Others techniques used to reduce spectroscopic interferences are matrix removal prior to analysis and the use of high resolution MS.

Most cases of non-spectroscopic interference refer to a matrix-induced change in signal intensity that is unrelated to the presence of a spectral component. They manifest themselves by signal suppression or enhancement; although most commonly by suppression. These interferences are mainly attributable to space charge effects. Space charge effects arise from the mutual repulsion of ions in the ion beam, which influence ion trajectories. Many cases of non-spectroscopic interference could be easily overcome by (a) use of suitable sample dilution step and (b) use of internal references or application of standard addition techniques or isotope dilution techniques instead of external standard calibration.

Matrix effects are quite general in that almost any contaminant with elevated concentrations will cause an effect. To reduce matrix effects the injector gas flow rate can be reduced in the ICP torch and ion lens modification can be utilized to enhance the through put of certain mass ranges thereby avoiding interfering matrix elements. It is always beneficial to avoid spectroscopic interference by matrix removal procedures when possible. The use of internal standards, while they do not directly reduce or eliminate matrix effects, can be used to compensate for some of the changes that may occur.

### 1.6.6 The coupling of HPLC-ICP-MS for the speciation of arsenic

The advantage of using HPLC is that the technique can separate arsenic species with the correct chromatographic conditions and the advantage of using ICP-MS as the detector of choice for the determination of arsenic is the wide range of samples that may be measured at concentrations in the ng $l^{-1}$ to μg $l^{-1}$ range. The coupling of HPLC with ICP-MS, as a detector, is now well established with an extensive number of publications supporting its popularity. Much work has been directed towards the separation of a greater numbers of species per analysis[82,127] with high speed separation[128] and improvement of limits of detection. For arsenic speciation detection limits as low as 0.005 ng of arsenic have been reported[129]. The molecular forms of arsenic commonly encountered in the environment are anionic, e.g. arsenate, MMA and DMA, cationic, e.g. the quaternary arsonium species AsB, AsCh and TeMA, or uncharged compounds at neutral pH, e.g. arsenite and TMAO. Arsenosugars present in marine algae are another commonly encountered group of arsenic-containing species. Their chromatographic behavior is dependent on the size of the molecule and the functional groups present in given solvent conditions. The simultaneous speciation of 17 arsenic species has been achieved using HPLC-ICP-MS with an anion exchange column and a gradient mobile phase within 900 seconds[130]. More commonly, fewer species are reported relying on the use of an anion exchange chromatography for the separation of neutral, anionic and cationic arsenic species independently[116,131, 132-134].

Anion column packing materials are often based on the copolymerization of styrene with divinylbenzene to produce a degree of cross-linking. The Hamilton PRP-X100 (strong anion exchange) is one of the most frequency employed. These columns are resistant over pH range of 1-13. This allows for a variety of eluents to be employed when establishing optimum separation conditions. High salt eluents such as sulphates and phosphates are good at providing ion-exchange chromatographic separation. However, when coupling HPLC to ICP-MS the salt content in the mobile phase needs to be less than 2% to reduce the risk of blocking the nebulizer and eroding the sampler and skimmer cones in the MS detector. Ion exchange chromatographic generally utilizes low concentrations of buffer, which help to reduce these problems and of those related to matrix interference effects. Although much work is based on salt buffers, nitric acid has been used successfully with less clogging of the sampler cone being reported[135].

Modifications to the chromatographic and ICP-MS system have been reported that can improve the sensitivity of detection. The modifications have included the use of various types of nebulizers, spray chambers and the introduction of alternative gases to the plasma. Two designs of spray chamber that are often used in commercial ICP-MS instrumentation, are the double-pass and the cyclonic. Research carried out into the performance of spray chambers[136] demonstrates that the transport efficiency and washout times for the cyclonic spray chambers are much more effective thereby improving signal:noise ratios and the overall performance of analyte detection. Cooling jackets around the spray chamber can also improve performance by reducing the solvent load to the plasma, particularly where organic solvents are employed in the mobile phase.

## 1.7 Aims of the study

This study focused on developing and applying a technique for total arsenic and arsenic species. Experiments ranged from laboratory experiments such as the sample extraction and detection experiment for arsenic species and apply to environmental sampling of sediment, water and biota in the vicinity of the Pak Pa-Nang River Basin.

The specific aims of this study are:-

- To develop suitable analytical methods for arsenic speciation analysis using methodology based on HPLC separation coupled with ICP-MS detection. By selection of suitable isolation techniques for arsenic species in a variety of samples, values of the various species under investigation at optimum conditions may be achieved to target the level of analytical performance required. The method to be subject to quality control and quality assurance.

- To use the analytical approach for the determination of arsenic species present in a variety of environmental samples from the high-risk arsenic-contaminated area, Pak Pa-Nang Estuary, Thailand. To carry out initial studies using HPLC-ICP-MS with extraction techniques for arsenic species in a variety of CRM and samples where appropriate.

- To assess the general water quality of the Pak Pa-Nang Estuary, which has not been studied to any great extent.

- To develop a conceptual model for arsenic transport from the arsenic contaminated area to the estuary and to define a new understanding the dynamics of arsenic movement between the various compartments of the Pak Pa-Nang River Basin.

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# Chapter 2

# Methodology for the Determination of Arsenic and Arsenic Species

## 2.1 Introduction

Many recent studies of arsenic have been focused on speciation because its toxicity differs greatly among its species[1]. Inorganic species, arsenite ($As^{III}$) and arsenate ($As^{V}$) are extremely toxic and have also been classified as being carcinogenic[2,3]. Organic arsenic species e.g., MMA and DMA have been classified as being cancer promoters[4]. Arsenobetaine (AsB), the major species found in organisms, is relatively non-toxic and can be excreted un-metabolised from the human body[5-8].

Research in this area has utilized instrumental techniques that include HPLC for separation purposes coupled with element-specific detectors such as ICP-MS[9-11], atomic absorption spectrometry (AAS)[12,13] , atomic emission spectrometry (AES)[14,15] and atomic fluorescence spectrometry (AFS)[16,17]. Coupling of HPLC with ICP-MS has received interest because it combines the efficient separation and detection of arsenic species[18-25]. The advantages of ICP-MS are its high sensitivity, its element-specific capability and its time-resolved mode that allows on-line real time analysis of the HPLC eluants.

The aims of this chapter are to develop and optimize a system of HPLC separation and ICP-MS detection together with the selective extraction techniques to determine species of arsenic in both reference materials and real samples. Their application to various

biota and sediment samples from the Tamar Estuary, UK (a pilot study) to validate the techniques was also performed.

## 2.2 Experimental

### 2.2.1 Instrumentation

**• ICP-MS instrumentation**

For total arsenic, ICP-MS measurements were performed using a VG PlasmaQuad 2 (VG Elemental, Winsford, Cheshire, UK). The combination of chlorine introduced via the sample matrix with argon from the plasma can give rise to the formation of $^{40}Ar^{35}Cl^{+}$, which interferes with the monoisotopic $^{75}As^{+}$. The problem was overcome by adding the molecular gas nitrogen to the nebulizer gas (4.5% v/v) according to the method approved by Hill et al.[26] A gas blender (Signal series 850, Signal, Camberley, Surrey, UK) was used for the nitrogen gas addition. An indium standard was used as an internal standard for all samples at a final concentration of 100 μg $l^{-1}$. The mass spectrometer was set to sample ion intensities (peak jumping option) at the analyzed mass m/z 75 ($^{75}As^{+}$). The operating conditions are given in Table 2-1. Additionally, the signal intensity is sampled at m/z 115 ($^{115}In^{+}$), used for internal standardization. The sample introduction is applied by a peristaltic pump at flow rate of 1.5 ml $min^{-1}$.

**Table 2-1 ICP-MS operating conditions used for the determination of 'total' arsenic in sample digests and extracts.**

| | |
|---|---|
| ICP-MS | PlasmaQuad 2 |
| Nebulizer type | Ebdon, high solids |
| Spray chamber | Double-pass, water cooled Scott type |
| Forward power | 1350 W |
| Gas flows/l $min^{-1}$ | |
| Nebulizer | 1.0 |
| Auxiliary | 1.25 |
| Coolant | 13 |
| $N_2$ addition | 4-4.5% v/v of Nebulizer gas flow |
| Isotope masses | $^{75}As$ and $^{115}In$ |

• **Hydride Generation-AAS instrumentation**

The technique used for the determination of dissolved inorganic arsenic ($As^{III}+As^{V}$) and total dissolved arsenic in water samples was hydride generation coupled with AAS. A Perkin-Elmer, FIAS 400 hydride generation flow injection system with a Perkin-Elmer, 4100 ZL AAS with Zeeman-effect background correction was used. Arsenic analysis by HGAAS was performed using the instrumental settings and conditions given in Table 2-2.

**Table 2-2 HGAAS operating conditions used for the determination of reducible arsenic.**

| | |
|---|---|
| AAS | Perkin-Elmer 4100 ZL |
| Lamp type, current | Electrodeless Discharge, 350 mA |
| Wavelength | 193.7 nm |
| Band width | 0.7 nm |
| Cell temperature | 900 °C |
| HG | FIAS 400 |
| Sample solution | $As^{3+}$ in 10% (v/v) HCl |
| Carrier solution | 10% (v/v) HCl |
| Reducing agent | 0.2% $NaBH_4$ in 0.05% NaOH |
| Purge gas | Argon |

• **HPLC instrumentation**

For chromatographic separation, a Waters 6000A chromatographic pump (Waters, Milford, MA, USA) was used with a 250x4.6 mm stainless steel column packed with 10 μm particle size Hamilton PRP-X100 anion-exchange resin. The column was protected by a 50x4.6 mm guard column packed with the same material. The sample solution was injected into a six-way injection valve (Rheodyne 7125) which was connected to the column. A pump (Waters Associates, series 6000) was used to supply pressure to achieve a reasonable flow rate in order to carry the solution through the column. The HPLC system was interfaced with the ICP-MS instrument using a Teflon capillary tubing (0.5 mm i.d.) to connect the column outlet directly with an inlet to the nebulizer. The specification of the system and operating conditions are given in Table 2-3. The ICP-MS was set to time-resolved data acquisition. Data for arsenic (m/z 75)

were recorded using the peak jumping acquisition and displayed as mass-intensity-time plots. The concentrations of each arsenic species were calculated using peak areas and were compared with standard solutions that were injected alternately. In addition, the spiking of sample extracts with known standards was also employed to take account of those matrix effects upon retention time and response factors.

**Table 2-3 HPLC specification and operating conditions for arsenic species.**

| Parameters | Experimental conditions |
|---|---|
| Column dimension | 250 x 4.6 mm |
| Guard column dimension | 50 x 4.6 mm |
| Packing material | Hamilton resin PRP-X100, 10μm particle size |
| Eluent flow rate | 1.5-2 ml $min^{-1}$ |
| Sample loop | 20, 100 μl |
| Competitive counter ion | Sulphate, Phosphate |
| Mobile phases | 5, 50 m mol $l^{-1}$ $Na_2SO_4$ pH 10-10.5* |
| | 2, 50 m mol $l^{-1}$ $H_3PO_4$ pH 7.5* |

*Adjusted with ammonia solution.

### 2.2.2 Chemicals and reagents for arsenic analysis in solid samples

All solutions were prepared with Milli-Q (18 MΩ.cm) water. All chemicals were of analytical or aristar grade and used without further purification. Arsenous acid, arsenic acid standards were prepared from a high purity stock solution of 996±2 mg As $l^{-1}$ (Aldrich, Gillingham, Dorset, UK). DMA standard solution was prepared from Cacodylic acid, sodium salt 98% (Sigma, Gillingham, Dorset, UK). AsB and MMA were used as stock solutions of 1,000 mg As $l^{-1}$ in terms of the element. Solutions of the compounds for daily use were prepared by appropriate dilution from the stock solutions. For arsenic speciation analysis in fish, shellfish and seaweeds AsB, DMA, MMA, and inorganic arsenic standards were prepared in 0.1 mol $l^{-1}$ $NH_4HCO_3$ (pH 8). For arsenic speciation analysis in sediments $As^V$, $As^{III}$, DMA and MMA standards were prepared in 0.25 mol $l^{-1}$ $H_3PO_4$. The $As^V$ and $As^{III}$ standards were prepared freshly each

time of analysis in order to avoid oxidation of $As^{III}$ to $As^{V}$. Indium and cesium stock standards were obtained from BDH laboratory supplies, Poole, UK. Trypsin (from bovine pancreas; 11,800 units $mg^{-1}$) powder and cellulase (from *Penicillium funiculosum*; 9.4 units $mg^{-1}$) enzyme were purchased from Sigma (Sigma, Gillingham, Dorset, UK). Hydrogen peroxide 37% was purchased from Merck, Poole, Dorset, UK. Nitric acid (Aristar), ammonium hydrogen carbonate (AnalaR) and sodium hydroxide pellets (AnalaR) were also purchased from Merck, Poole, Dorset, UK.

An analytical column and a guard column were packed with Hamilton PRP-X100, 10µm particle size resin purchased from Phenomenex, Cheshire, UK. Columns were packed in-house using a wet packing technique in 5 mmol $l^{-1}$ $Na_2$ $SO_4$, pH 10 at 2000-3000 pounds per square inch pressure.

Glassware and plastic centrifuge tubes were pre-cleaned by soaking for at least two days in 5% Decon 90 (Merck) in Milli Q water followed by soaking for two days in 10% v/v nitric acid made up with Milli Q water and then rinsed with Milli Q water prior to use.

### 2.2.3 Certified reference materials and samples

The purpose of using a certified reference material of similar matrix to that of the sample under study is that the concentration of the analyte is known together with the major characteristic of the matrices. Since analytical errors can arise from various sources, including improper analytical technique, interference by matrix components and incomplete digestion or extraction of the analyte, using certified reference materials will assist in identifying whether or not the determination has accuracy and is reliable. This is sometimes termed as a 'validation' step.

The certified reference materials (CRM), DORM 1 (dogfish muscle), TORT 2 (lobster hepatopancreas) and PACS 1 (marine sediment) were obtained from the National Research Council, Ottawa, Canada. LGC 6137 estuarine sediment reference material was obtained from the Laboratory of the Government Chemist, Middlesex, United Kingdom. Kelp powder was an in-house standard material obtained from the Laboratory University of Plymouth. The certified reference materials and the standard material were used to validate the methods and to ensure the analytical quality control.

After the optimization and validation of the technique using reference materials (see section 2.3), an analysis for 'total' and 'speciation' of arsenic in some real samples, mussel, cockle, green seaweed, brown seaweed and sediment from the Tamar Estuary, UK (Figure 2-1) was performed. The samples were collected in October 2000 and were prepared immediately in the lab after the sampling. The main objectives of this sampling were as follows:

1. To evaluate the sample digestion technique for total 'available' arsenic in Teflon bombs by microwave digestion using nitric acid and hydrogen peroxide.

2. To evaluate the arsenic speciation methods employing a HPLC separation and ICP-MS detection of arsenobetaine, DMA, MMA, inorganic arsenic, $As^{III}$ and $As^{V}$ species in shellfish, seaweed and sediment samples.

3. To study the total concentration of arsenic and arsenic species in those samples that were collected.

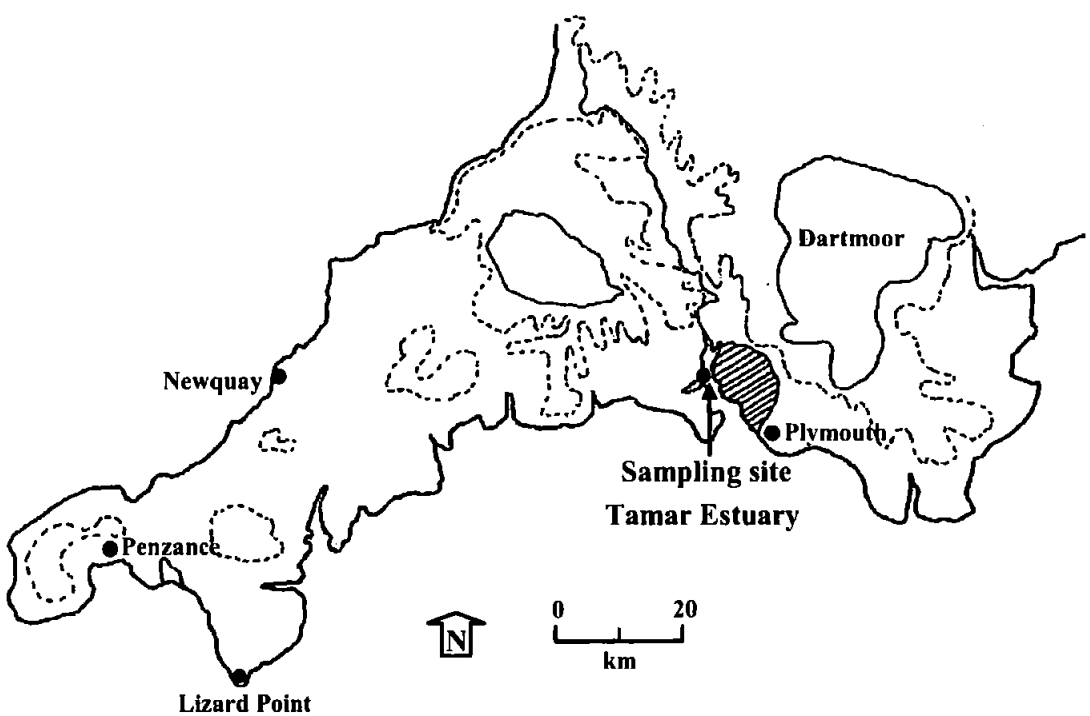


**Figure 2-1 Pilot study sampling location in the Tamar Estuary; the sampling was taken in October, 2000.**

• **Sample preparation**

The different samples were collected in separate bags, stored in a cool box and then returned as quickly as possible to the laboratory. Mussels and cockles were cleaned with Milli Q water and separated from shell. Green seaweed and brown seaweed were rinsed with Milli Q water and cut to small pieces. Sediment samples were put into a beaker. All the samples were pre-weighed and then frozen at -40 °C for 12 hrs in a freezer and then placed in a freeze drier for 48 hrs. The dried mussel, cockle, green seaweed, brown seaweed and sediment samples were again weighed then ground using an agate pestle and mortar to a fine powder and then sieved using a nylon sieve (180 μm sieve mesh). The samples were then stored in brown bottles and placed in a desiccator in order to avoid exposure to light and moisture until required for analysis.

### 2.2.4 Sample digestion procedures for total arsenic in solid samples

In the determination of total element concentrations it is necessary to utilize a sample decomposition technique that will ensure that the analyte of interest is brought into solution and remains in solution. The analyte must be stable and any chemicals used do not cause instrumental interference. The practice of microwave digestion has been comprehensively reviewed[27]. Nitric acid (69%-azeotropic) has a boiling point of 122 °C and in order to adjust the oxidizing potential of $HNO_3$, by means of elevating the temperature, closed vessel microwave conditions are used[28]. The decomposition process is further assisted by the addition of hydrogen peroxide and the oxidizing power of $HNO_3$ which increases with higher concentrations. Once complete digestion has been obtained, the elements of interest remain in solution and can be determined by the chosen method of detection.

The digestion for total arsenic in the biological and sediment samples employed nitric acid and hydrogen peroxide. The samples were digested using microwave-assisted digestion in Teflon bombs as shown in the procedure in Table 2-4. The period of heating was not more than 3 minutes for 4 bombs in each heating period in order to avoid explosion from over pressure in the digestion bombs. If after the first 3 minutes of heating, the solutions were still clear or yellow, another period of heating was under taken. A completed digest of a biological material was usually dark in colour.

**Table 2-4 Acid digestion procedure for total available arsenic.**

| Step | Procedure |
|---|---|
| 1 | Weigh 0.25 g freeze dried sample directly into a digestion bomb |
| 2 [a] | Add conc. (69%) Nitric acid (4 ml) and hydrogen peroxide 37% (1 ml) then cap the bomb loosely and leave overnight for pre-digestion |
| 3 [b] | After pre-digestion, cap the bomb securely and heat in a suitable microwave oven (700 w) at medium power for about 3 minutes (for 4 bombs); follow with another period of heating to complete the digestion |
| 4 | After cooling transfer the digest to a 50 ml volumetric flask, spike with the internal standard and dilute to volume using 2% nitric acid |
| 5 | The digest is ready for analysis |

[a] For some materials, heating at this early stage may lead to an explosion, hence the need for a cool pre-digestion stage; [b] A completed digest of a biological material is usually dark in colour, if the digest is still yellow, it may be necessary to heat for a longer period. Occasionally it may be necessary to add a further aliquot (1 ml) of nitric acid in addition to the heating to ensure complete digestion.*This digestion is also suitable for other trace metal determination, such as Cu, Ni and Pb.

### 2.2.5 Sample extraction procedures for arsenic species in solid samples

**• Enzymatic extraction for arsenic species in biological samples**

Acidic microwave digestions can destroy information on species. Where speciation analysis is to be performed digestion procedures that retain the chemical form of the compound must be employed. The choice of a suitable enzyme for the sample matrix, where the cell contents can be released into solution unchanged, is one way in which this can be achieved. Enzymatic extractions are widely reported in the literature with effective extraction of the species under consideration[11,21,29,30]. Optimum conditions of pH and temperature must be employed, as enzyme activity is sensitive to these parameters.

**- Fish and shellfish samples**

Trypsin is an enzyme that is well known as a protein degrader. It breaks down dietary protein molecules to their component peptides and amino acids. Trypsin has been found

to work well in a slightly alkaline environment, about pH 8 at 37 °C (human body temperature). Arsenic species in the marine animal samples were extracted using trypsin enzyme in 0.1 mol $l^{-1}$ $NH_4HCO_3$, (pH 8). The extraction procedure is shown in Table 2-5.

**Table 2-5 Enzymatic extraction procedure for arsenic species in fish and shellfish.**

| Step | Procedure |
|---|---|
| 1 | Weigh 0.25 g freeze dried sample and 0.1 g trypsin directly into a Potter homogenizer |
| 2 | Add 0.1 mol $l^{-1}$ $NH_4HCO_3$ (10 ml) and homogenize with the sample then transfer to a plastic centrifuge tube |
| 3 | Add another 0.1 mol $l^{-1}$ $NH_4HCO_3$ (10 ml) to rinse the homogenizing tube then transfer to the same centrifuge tube |
| 4 | Cap the tube and leave in a shaking water bath at 37 °C for 12 hrs |
| 5 | Centrifuge the sample at 2500 rpm for 20 min |
| 6[a] | Pour the extract into a volumetric flask (25 ml) spike with Cs internal standard solution and dilute to volume with 0.1 mol $l^{-1}$ $NH_4HCO_3$ |
| 7 | The extract is ready for analysis (the extract can be kept in darkness at 4°C for no longer than 1 week) |

[a] Keep the residual solid for residue-arsenic analysis.

- **Seaweed samples**

The use of a cellulase enzyme extraction procedure to maintain the integrity of the arsenic species that are efficiently removed from plant samples, has attracted considerable interest in the last few years. The activity of the enzyme depends primarily on the pH and temperature of the reaction system. Basically, cellulases can be called acid-cellulase because the pH range from 4.5 to 5.0 is required for optimum enzymatic activity. Tight temperature control is required to obtain reproducible results.

To break down the cellulose cell walls in seaweed samples, cellulase enzyme was used in the extraction. The enzyme worked well in 0.1 mol $l^{-1}$ $CH_3COONH_4$, (pH 5). The extraction procedure is shown in Table 2-6.

**Table 2-6 Enzymatic extraction procedure for arsenic species in seaweed samples.**

| Step | Procedure |
|---|---|
| 1 | Weigh 0.5 g freeze-dried sample and 0.5 g cellulase directly into a Potter homogenizer |
| 2 | Add 0.1 mol $l^{-1}$ $CH_3COONH_4$ (10 ml, pH 5) and homogenize with the sample then transfer to a plastic centrifuge tube |
| 3 | Add another 0.1 mol $l^{-1}$ $CH_3COONH_4$ (10 ml, pH 5) to rinse the homogenizing tube then transfer to the same centrifuge tube |
| 4 | Cap the tube and leave in a shaking water bath at 37 °C for 12 hrs |
| 5 | Centrifuge the sample at 2500 rpm for 20 min |
| 6[a] | Pour the extract into a volumetric flask (25 ml), spike with In internal standard solution and dilute to volume with 0.1 mol $l^{-1}$ $CH_3COONH_4$ |
| 7 | The extract is ready for analysis (the extract can be kept in darkness at 4°C for no longer than 1 week) |

[a] Keep the residual solid for residue arsenic analysis

- **Phosphoric acid extraction for arsenic species in sediment samples**

Phosphoric acid was used successfully to extract arsenic species from sediments in this study. The extraction process followed previous studies of the arsenic speciation in soils and sediments[22,25]. An organic microwave digester system, Synthewave 402 (Prolabo, Fontanay-Sous-Bois Cedex, France) was used in the extraction. The system was a focused microwave in an open quartz flask with both power, and heating time adjustable. During the extraction, a glass-stirring paddle was used to stir the sediment accompanied with the flask rotation. To achieve the highest extraction efficiency, the LGC 6137 estuarine sediment reference material was used as a sample in the optimization experiment. Samples (0.5 g) were extracted using the process shown in Table 2-7. The result of the optimization is shown in section 2.3.2.

Table 2-7 **Phosphoric acid extraction procedure used to determine arsenic species in sediment samples.**

| Step | Procedure |
|---|---|
| 1 | Weigh 0.5 g freeze dried sediment sample directly into a beaker |
| 2 | Add 1 mol $l^{-1}$ $H_3PO_4$ (25 ml) and stir with a glass rod then transfer to the extraction vessel |
| 3 | Place the vessel into the microwave digester and heat at 30-60 W for 10-20 minutes |
| 4 | Let the sample cool down then transfer to a centrifuge tube |
| 5 | Centrifuge the sample at 2500 rpm for 20 min |
| 6 | Pour the extract into a volumetric flask (100 ml) spike with internal standard solution and dilute to volume using Milli Q water |
| 7 | The extract is ready for analysis |

### 2.2.6 Arsenic mass balance for the analysis of biota and sediment samples

A mass balance is a quality index of the extraction technique. In practice, some residual solid is always found after the enzymatic extraction or phosphoric acid extraction process. Some arsenic mass remains within the residual solid and this has to be measured after using a nitric acid digestion. To obtain full data for the mass balance calculations, the determination of total arsenic concentration in the sample digests and extracts, the sum of the species in the extracts together with total arsenic concentrations in any residues must be available. In general, the sum of concentrations of arsenic species (AsB, DMA, MMA and inorganic arsenic) combined with the arsenic, if any, retained in the residual solids from the enzyme or phosphoric extraction, should be equal to the concentration of total arsenic. An outline of this mass balance approach is shown in Figure 2-2.

### 2.2.7 Preparation for dissolved arsenic and suspended particulate matter (SPM) analysis

For dissolved arsenic analysis, collection of water samples was performed using 1 litre acid-washed plastic bottles. This was then filtered using an acid-washed Millipore filter

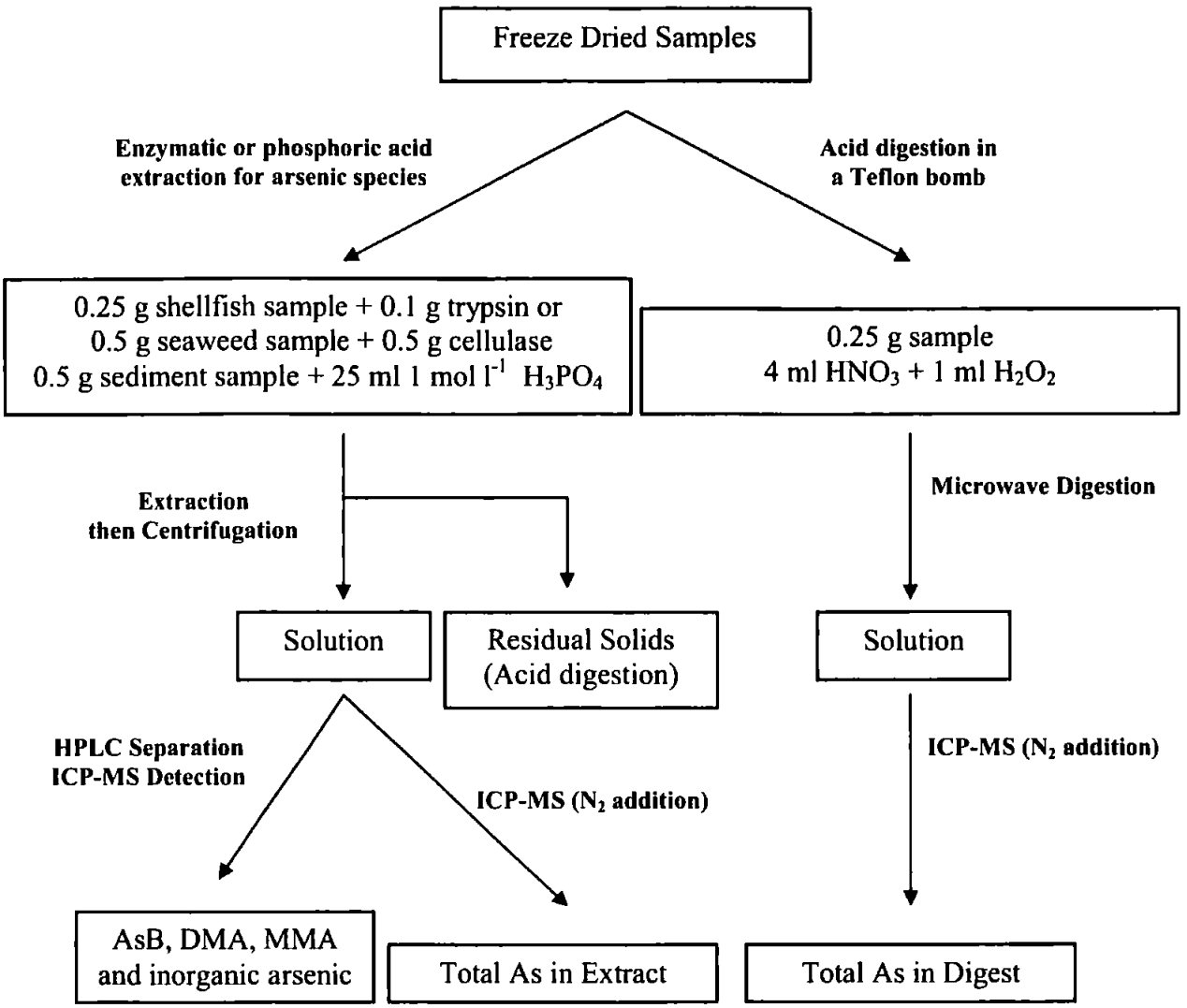


**Figure 2-2 Arsenic mass balance in biota and sediment sample analysis.**

with 0.45 μm pore size or a pre-weighed glass fibre filter to provide a 250 ml water sample. The filtrate (250 ml) was then acidified with 0.25 ml conc HCl and stored in a fridge prior to analysis. The filter, with SPM was then stored in a plastic container and freeze dried for total arsenic and arsenic species analysis. Acidified water sample analysis for dissolved inorganic arsenic and total dissolved arsenic procedures are shown in Table 2-8 and 2-9, respectively.

**Table 2-8 Procedure to determine dissolved 'inorganic' arsenic in water samples.**

| Step | Procedure |
|---|---|
| 1 | Pipette 4 ml of acidified water sample to a 10 ml test tube |
| 2 | Add conc. HCl (0.5 ml), 5% KI (0.25 ml) and 5% Ascorbic acid (0.25 ml) then shake it well |
| 3 | Let the sample equilibrate for 45 minutes |
| 4 | The sample is ready for analysis by HGAAS |

**Table 2-9 Procedure to determine 'total' dissolved arsenic in water samples.**

| Step | Procedure |
|---|---|
| 1 | Pipette 9.9 ml of acidified water sample to a 10 ml test tube |
| 2 | Add conc. (69%) Nitric acid (0.1 ml) and $K_2S_2O_8$ (0.1 g) then heating in boiling water for 20 minutes. |
| 3 | Let the sample cool down then add $NH_3OHCl$ (0.1 g) |
| 4 | Pipette 4 ml of the sample from step 3 to another 10 ml test tube |
| 5 | Add conc. HCl (0.5 ml), 5% KI (0.25 ml) and 5% Ascorbic acid (0.25 ml) then shake it well |
| 6 | Let the sample equilibrate for 45 minutes |
| 7 | The sample is ready for analysis by HGAAS |

## 2.3 Optimization of the methods

### 2.3.1 Digestion conditions for total arsenic in biota and sediment samples

To optimize the digestion time for total arsenic using the microwave-assisted digestion in Teflon bomb, standard reference materials, Tort 2 (Lobster hepatopancreas), Kelp (Seaweed) and LGC 6137 (Estuarine sediment) were digested using the process shown in the Table 2-4. A series of heating times, first and second heating periods, has been compared using, (a) 2+1 series (heating two minutes and leave the sample to cool down for 15 minutes then heating again for one minute), (b) 3+2 series (heating three minutes and leave the sample to cool down for 15 minutes then heating again for two minutes) and (c) 3+3 series (heating three minutes and leave the sample to cool down for 15

minutes then heating again for three minutes). The result of the optimization is shown in Figure 2-3.

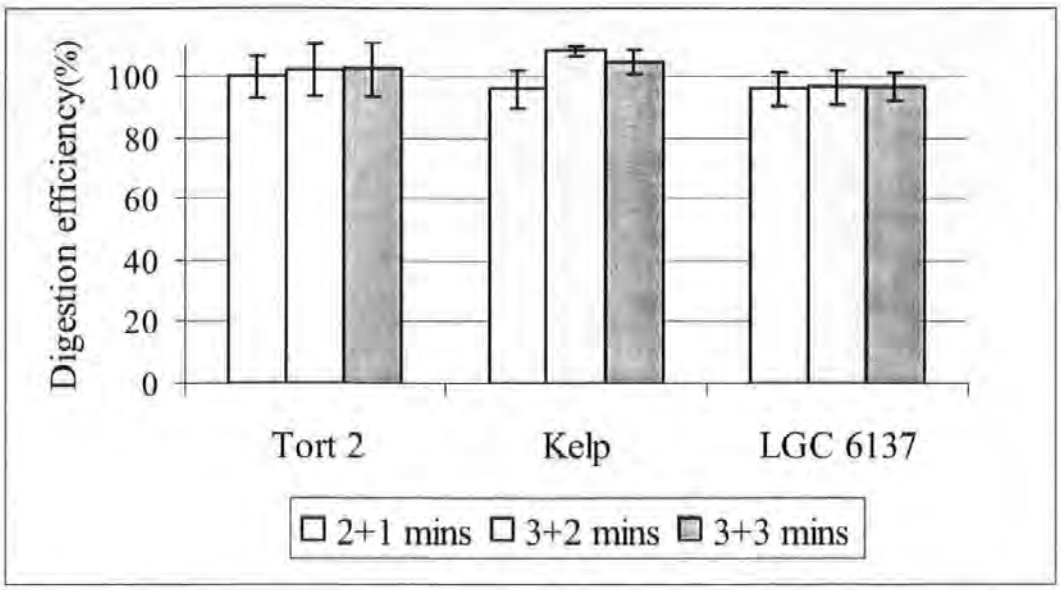


**Figure 2-3 Digestion efficiency for total arsenic from reference materials using the microwave-assisted digestion in a Teflon bomb and different digestion times (n=3).**

Using the microwave-assisted digestion in Teflon bombs for 'total' arsenic in the CRMs was successful. Good digestion efficiencies, from 96 to 108% were observed using a variety of digestion times. The digestion efficiencies with the moderate time, 3+2 minutes, show slightly greater values than the shorter time, 2+1 minutes, for every reference material, especially in Kelp, which is a macro algae. This suggested that the cellulose in the Kelp cell wall required higher temperatures and/or time to break down. About the same digestion efficiencies were observed using a longer time, 3+3 minute digestions compared with the 3+2 minute digestion. It is noted that use of the sealed-bomb technique was to ensure that volatile arsenic species, if formed, were not lost from the digests.

A dark green colour was observed in Tort 2 digests with the 3+2 and 3+3 minute digestion times, which indicated a complete digestion for a biological material as stated earlier. However, the digest colour of Tort 2 with the 2+1 minute digestion was an amber green, which indicated an incomplete digestion. For Kelp and LGC 6137, a dark brown colour was observed in the complete digestions using 3+2 and 3+3 minute digestion times. A yellow colour was observed in an incomplete digest with the 2+1 minute digestion period. High recoveries of arsenic were achieved from CRMs using

the digestion series 3+2 minutes and provided complete digestion with low risk of volatile arsenic species loss. This time series was adopted and applied to the digestion of real samples.

### 2.3.2 Optimization of extraction conditions for arsenic speciation in sediment analysis

The LGC 6137 estuarine sediment reference material was used as a sample in the optimization experiment. Samples (0.5 g) were extracted under various microwave-heating conditions as shown in Table 2-10 (the extraction process is described in Table 2-7). The results show good extraction efficiency, ranging from 78-98% of total arsenic in extracts compared with the certified value. The conditions of 45W power for a 20 minute extraction period was selected for the final extraction procedure of this work because it gave the highest efficiency (96-98%), with a lower risk of species modification compared with 60W for 20 minute extraction period.

**Table 2-10 Optimization of phosphoric acid extraction for total arsenic in LGC 6137 (12.4±1.8 μg $g^{-1}$ As) (n=3).**

| Power (Watts) | Time (Minutes) | Extraction efficiency (%) |
|---|---|---|
| 30 | 10 | 78-84 |
| 45 | 10 | 82-84 |
| 60 | 10 | 83-85 |
| 30 | 20 | 91-92 |
| 45 | 20 | 96-98 |
| 60 | 20 | 96-98 |

-The heating power from 45W induces a temperature of 120 °C in the extraction process.

Using the later-confirmed chromatographic conditions for arsenic species measurement in sediment extracts (section 2.3.3), the stability of the arsenic species under the extraction condition, 45W for 20 minutes, was determined by spiking those arsenic species of interest along side the estuarine sediment reference material. The extracts were then analysed for these arsenic species and their recoveries calculated for each arsenic species, to ensure the integrity of those species after the extraction. The results

showed good stability for all the species after the extraction with recovery more than 90% for $As^{III}$, $As^{V}$, MMA and DMA species. The most variability was found for $As^{III}$ with a decreased value of between 4-8% of the spiking value. A concordant increase in $As^{V}$, suggested that some $As^{III}$ was oxidized to $As^{V}$ during the extraction and measurement process. A 1% increase and 2% decrease were found in MMA and DMA species, respectively which, considering the analytical uncertainty was felt to be acceptable.

### 2.3.3 Choice of chromatographic conditions

The chromatographic separation of arsenic species was achieved using a high capacity anion-exchange-based column with sulphate and phosphate-base mobile phases. The main factors that influence the separation of arsenic species using High Performance Liquid Ion Chromatography are the values of pKa of the species, and pKa pH, buffering capacity and ionic strength of the mobile phase. Changes in the analytes' ionic charge, by changing pH, will influence the mobility of the analyte species. Increasing the pH will enhance the ionization of arsenic, arsenous, dimethylarsinic and monomethylarsenic acids. However, the extent of ionization can vary considerably between the acids considered (Figure 2-4)[31].

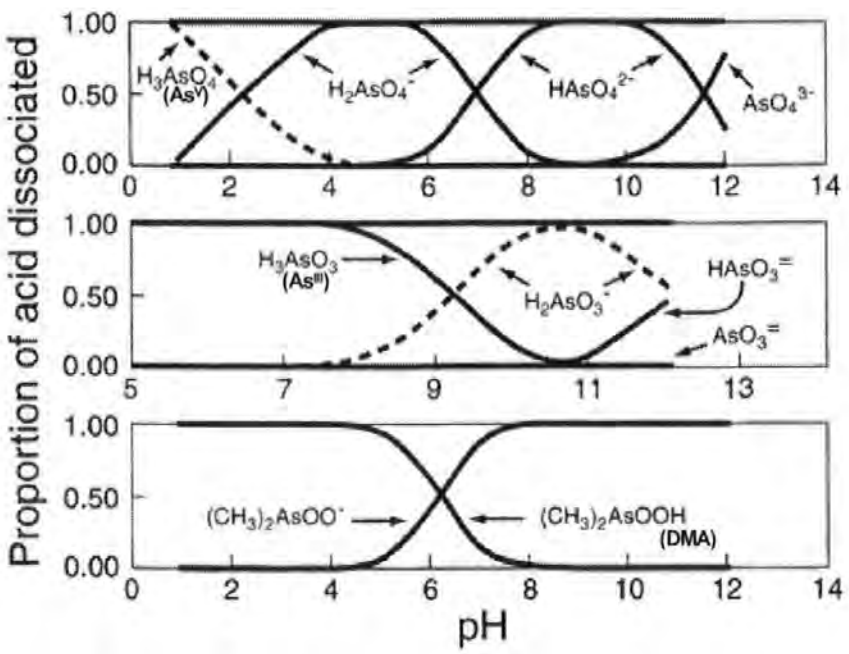


**Figure 2-4 Effect of solution pH on the proportion of acid that is dissociated, based on the acid $pK_a$ values (Source: Naidu, et al. 2000)[31].**

As stated, analyte mobility/retention is a major factor in determining separation efficiency and selectivity. Factors such as pH, ionic strength, and the temperature can be manipulated to improve separations through changes in analyte mobility[31]. If the composition of mobile phase and temperature is controlled during a separation, it is sound that the marked differences in the retention time is related to the dissociation and hence charge on the arsenic species because of its association/attraction to the positive charge on the stationary phase. For example, DMA is fully dissociated above pH 8 (Figure 2-4) and carries a single charge. Similar to DMA, 97% of arsenic acid is present in its, $HAsO_4^{2-}$, at pH 8, ionic form although the nature and proportion of the conjugate acid-base pair $H_3AsO_4/H_2AsO_4^-$ and $H_2AsO_4^-$ / $HAsO_4^{2-}$ varies with increasing pH [31]. At pH 5, all the neutral molecule is dissociated into the species $H_2AsO_4^-$ while at pH 10, over 97% is present as $HAsO_4^{2-}$ and less than 3% as $AsO_4^{3-}$ (Figure 2-4). In contrast to $As^V$ and DMA, the retention time for $As^{III}$ is shorter at pH 7.5 to 8 because arsenious acid is largely present as the uncharged species, above 94% (Figure 2-4). This would allow arsenous acid to pass through the column relatively unhindered, eluting close to the solvent front. A similar effect occurs with AsB at pH 10.

The pH of mobile phases selected for this work was based on $pK_a$ values, shown in Table 2-11. The different charges possible on the arsenic species of interest is shown to be pH dependent. The presence of $SO_4^{2-}$ and $HPO_4^{2-}$ in the eluents (used respectively for biota extracts at pH 10.2 and sediment extracts at pH 7.5) is for competition of sites on the stationary phase. Optimization is therefore directed towards the concentrations and gradient periods used of the sulphate and phosphate components.

**Table 2-11 $pK_a$ values for inorganic and organic arsenic[32].**

| Arsenic compound | | $pK_a$ value |
|---|---|---|
| Arsenious acid ($As^{III}$) | $HAsO_2 \rightarrow AsO_2^-$ | 9.23 |
| Arsenic acid ($As^V$) | $H_3AsO_4 \rightarrow H_{(3-n)}AsO_4^{n-}$ | 2.20, 6.97, 11.53 |
| MMA | $CH_3AsO(OH)_2$ | 3.6, 8.2 |
| DMA | $(CH_3)_2AsO(OH)$ | 1.28, 6.2 |
| AsB | $(CH_3)_3As^+CH_2CO_2^-$ | 2.18 |

n=1,2 or 3.

• **Optimization of HPLC for shellfish and seaweed sample extracts**

Figure 2-5a shows the chromatograms of four standard arsenic species, AsB, DMA, MMA and inorganic As, respectively. This separation was achieved successfully using the anion-exchange resin-based stationary phase column. The analysis was complete in 900 seconds using two concentrations of mobile phase $Na_2SO_4$, **a**: 5m mol $l^{-1}$ and **b**: 50 m mol $l^{-1}$, pH 10. The mobile phase elution was programmed as three steps following:

1. Isocratic elution — Mobile phase (a) for 360 seconds
2. Step gradient — Mobile phase (b) for 180 seconds
3. Re-equilibrate — Mobile phase (a) until the finish

AsB and DMA were eluted with mobile phase **a** in step one then MMA and inorganic As were eluted when the mobile phase was switched to **b** in step two. Step three was a re-equilibration step, which prepared the column for the next injection. The elution of all four species was possible using one concentration of mobile phase but, if concentration **a** was selected MMA and inorganic arsenic took an unacceptably long time to be eluted. On the other hand if concentration **b** was selected all the four species were eluted out together within 300 seconds without separation.

The separation of AsB and DMA was improved as the concentration of mobile phase **a** was decreased but DMA took a longer time to elute and the peak was broad if the concentration of mobile phase **a** was too low. The separation of MMA and inorganic arsenic was not dependent upon the concentration of mobile phase **b** but upon the running time of mobile phase **a** before switching to mobile phase **b**. While MMA and inorganic arsenic are both retained on the column when the low concentration eluent **a** is run, the MMA under these conditions migrates ahead of the inorganic arsenic on the stationary phase. Both were separated well as the running time of mobile phase **a** in step 1 was increased (Figure 2-6).

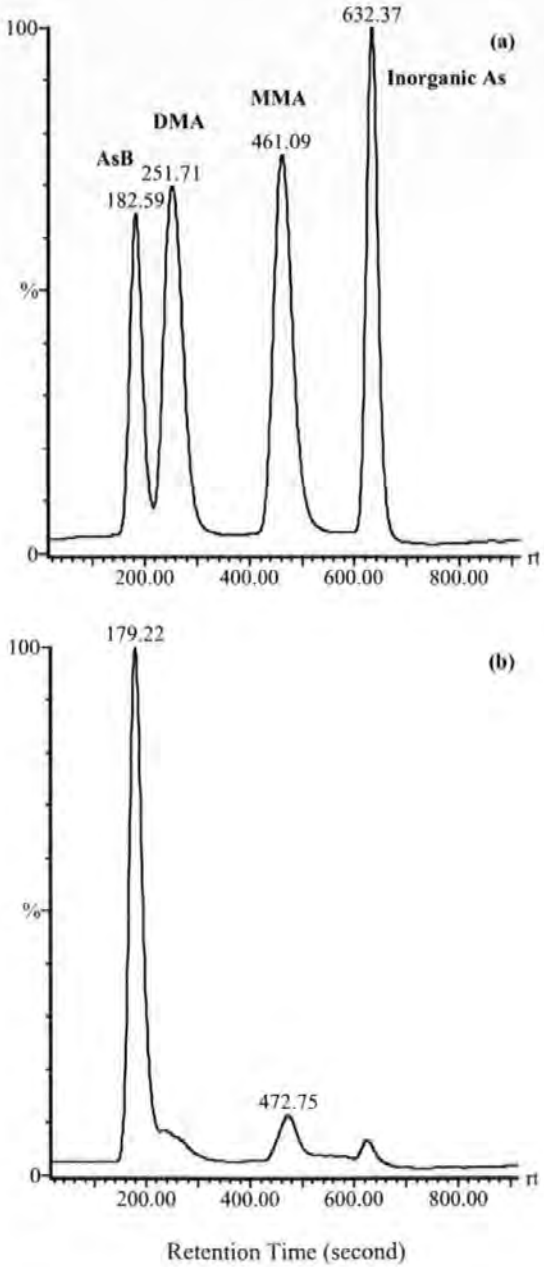


**Figure 2-5 Chromatograms showing arsenic species from aqueous standard solutions and CRM shellfish extract solutions; a: standard AsB, DMA, MMA and $As^{V}$ 200 ppb As in each species, b:TORT 2 extract using extraction procedure shown in Table 2.5.**

The concentration of mobile phase **b** was selected as a compromise in order that MMA and inorganic arsenic may be eluted in a short time period and so that the concentration was not high enough to cause blockage of the ICP-MS sampling cone and torch injector by sodium salts from the mobile phase.

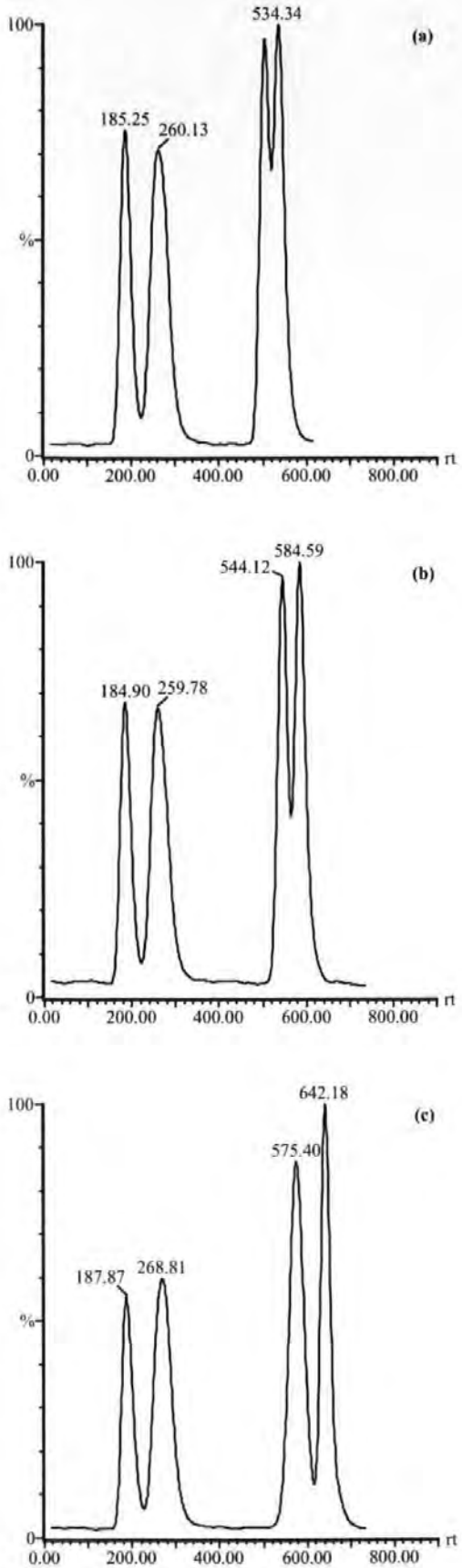


**Figure 2-6 Chromatograms of AsB, DMA, MMA and $As^{V}$ 200 ppb As in aqueous solutions using difference isocratic running times (step one); a:180 sec, b:240 sec, c:300 sec.**

• **Optimization of HPLC for sediment samples extracts**

This separation was achieved successfully using an anion-exchange resin-based stationary phase column. The analysis was complete in 900 seconds using two concentrations of mobile phase $H_3PO_4$ **a**: 2m mol $l^{-1}$ and **b**: 50 m mol $l^{-1}$, pH 7.5. The mobile phase elution was programmed as three steps following:

1. Isocratic elution — Mobile phase (a) for 360 seconds
2. Step gradient — Mobile phase (b) for 180 seconds
3. Re-equilibrate — Mobile phase (a) until the finish

Four standard arsenic species, $As^{III}$, DMA, MMA and $As^{V}$ (100 ppb As in each species) were prepared and injected into the HPLC-ICP-MS system. A typical chromatogram is shown in Figure 2-7. This is a similar process to the chromatographic approach for arsenic species in biological samples. $As^{III}$ and DMA were eluted with mobile phase **a** in step one then MMA and $As^{V}$ were eluted when the mobile phase was switched to **b** in step two. Step three was to re-equilibrate the system, which prepared the column for the next injection.

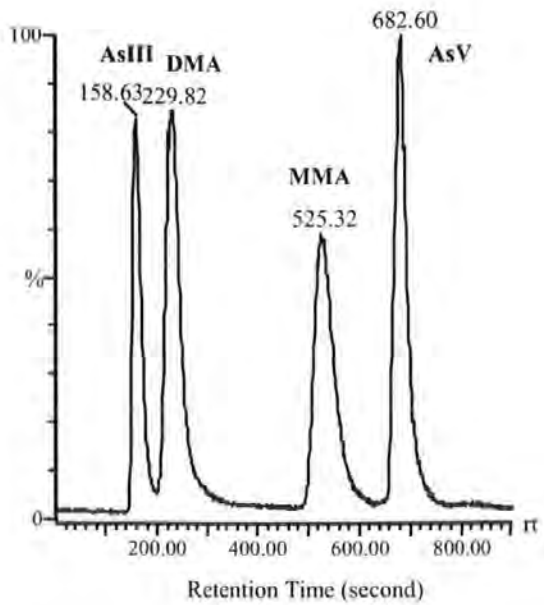


**Figure 2-7 Chromatogram of arsenic standard species using optimized conditions for sediment extracts; $As^{III}$, DMA, MMA and $As^{V}$, 100 ppb As in each species.**

### 2.3.4 Results of the analysis of CRMs and real sample extracts

**• Certified and in-house reference material analysis for total arsenic**

The digestion technique was optimized and validated for total arsenic. Analytical performance for 'total nitric available' arsenic was based on the use of standard reference materials, DORM 1, TORT 2, Kelp, LGC 6137 and PACS-1. These results are shown in Table 2-12. These reference materials were chosen to represent fish, shell fish, seaweed and sediment samples. A good digestion efficiency was found for DORM 1, TORT 2, Kelp and LGC 6137 being 96-107%. The exception was PACS-1 being only 44%. Hence, this matrix cannot be completely digested by nitric acid and hydrogen peroxide, and only a lower percentage arsenic recovery was observed. Near full arsenic concentration can be obtained using an aqua-regia digest. This does however, give an indication of the nitric acid 'available' arsenic. The occluded arsenic (matrix-trapped) would only become available over longer, geological, time periods compared with the more readily available biological time periods which would correlate better with toxicity assessments.

**Table 2-12 Certified and in-house reference material analyses for total arsenic, mean ± standard deviation (n=3).**

| Materials | Characteristic | Certified value ($\mu g\ g^{-1}$) | Concentration obtained ($\mu g\ g^{-1}$) | Digestion efficiency (%) |
|---|---|---|---|---|
| DORM 1 | Dogfish mussel | 17.7±2.1 | 17.4±1.0 | 98 |
| TORT 2 | Lobster hepatopancreas | 21.6±1.8 | 23.1±1.1 | 107 |
| Kelp* | Seaweed | (44±1.1) | 45±0.7 | 102 |
| LGC 6137 | Estuarine sediment | 12.4±1.8 | 11.9±1.1 | 96 |
| PACS-1 | Marine sediment | 211±11 | 93±17 | 44 |

*Kelp (*Ascophyllum nodosum*) –in-house reference material, reference value for 'total' arsenic = 44±1.1 $\mu g\ g^{-1}$ dry weight[33].

• **Certified reference material analysis for arsenic species**

- **Enzymatic extraction**

To measure the performance of the extraction techniques for arsenic species that have been used in this study, DORM 1 and TORT 2 were extracted using the trypsin enzyme. Total arsenic in the extracts from those CRMs were measured using $N_2$-ICP-MS. High arsenic extraction efficiency values of 97 and 108% were found respectively. Integrity of arsenic compounds in extracts is important as stated earlier. The lack of suitable CRMs for certain sample types containing measured arsenic compounds is still a problem for some speciation studies at present. One way to solve the problem is by comparing the results of arsenic compounds in a CRM that have been analyzed by many different groups of researchers using different extraction and detection techniques. These results are shown in Table 2-13.

For DORM1, AsB was the major arsenic compound found in this work with a concentration of 15.4 $\mu g\ g^{-1}$. That is in good agreement with the literature concentration range of 14.5-16.5 $\mu g\ g^{-1}$. The only minor component found in this work was DMA, which was also found by Goessler et al. [23] within the same range of concentration. MMA and inorganic arsenic have been reported in some studies as less than 0.05$\mu g\ g^{-1}$ to reflect their LODs and these species were not detected in this work.

For TORT2, the chromatogram is shown in Figure 2-5b. Two other studies have been found in the literature to compare with this study. AsB was in good agreement as the major species within the literature concentration range 16.6-22.3$\mu g\ g^{-1}$ and within the same statistical limits observed by Lamble and Hill[12]. MMA and inorganic arsenic were detected as trace components in this work with concentrations of 2 and 0.6 $\mu g\ g^{-1}$, respectively. The trace compounds for DORM 1 and TORT 2 in the literature were slightly different, possibly due to the detection limits for the different techniques used.

• **CRM analyses for validation in Tamar and Pak Pa-Nang studies**

In all 'real' sample analyses, the CRMs, DORM 1, TORT 2, Kelp, LGC 6137 were chosen to validate the digestion, extraction and instrumental techniques for arsenic and species measurement. The CRMs were analysed repeatedly throughout and Table 2-14 shows the overall accuracy and precision of the CRM analyses during the period of study.

**Table 2-13** **Concentrations of arsenic compounds (μg As $g^{-1}$ dry mass basis) in DORM 1 and TORT 2, mean ± standard deviation (n=3).**

| CRM | AsB | DMA | MMA | $As^{III}$ | $As^{V}$ | Extraction method | Analysis technique | Reference. |
|---|---|---|---|---|---|---|---|---|
| DORM 1 | 14.7 | NP | <0.03 | <0.05 | <0.05 | Chloroform-methanol-water | HPLC-ICP-MS | 34 |
| | 15.1±0.6 | NP | NP | NP | NP | Chloroform-methanol-water | HPLC-ICP-MS | 21 |
| | 16.1±0.4 | NP | NP | NP | NP | trypsin | HPLC-ICP-MS | 21 |
| | 16.5±0.6 | NP | NP | NP | NP | methanol-water | HPLC-MO-HGAAS | 35 |
| | 14.5±1.5 | ND | ND | ND | | trypsin | HPLC-MD-HGAAS | 12 |
| | 15.6±0.7 | 0.49 | <0.03 | <0.03 | <0.03 | methanol-water | HPLC-ICP-MS | 23 |
| | 15.4±0.6 | 0.6±0.05 | ND | ND | | trypsin | HPLC-ICP-MS | This work |
| TORT 2 | 16.6 | 1.6 | <0.03 | <0.05 | <0.05 | Chloroform-methanol-water | HPLC-ICP-MS | 34 |
| | 22.3±1.2 | ND | ND | 2±0.2 | | trypsin | HPLC-MD-HGAAS | 12 |
| | 20.8±1.4 | ND | 2±0.1 | 0.6±0.04 | | trypsin | HPLC-ICP-MS | This work |

ND=Not detectable
NP=Not performed
HPLC-MO-HGAAS = High Performance Liquid Chromatography-Microwave-assisted Oxidation-Hydride Generation Atomic Absorption Spectrophotometry
HPLC-MD-HGAAS = High Performance Liquid Chromatography-Microwave Digestion-Hydride Generation Atomic Absorption Spectrophotometry

**Table 2-14 CRM analyses for 'total' arsenic and species using acid digestion, phosphoric extraction and enzymatic extraction, mean ± standard deviation (n=number of CRM replicates).**

| CRM | Characteristic | Sample match | Certified value ($\mu g\ g^{-1}$) | Concentration obtained ($\mu g\ g^{-1}$) | n | Digestion/Extraction efficiency (%) |
|---|---|---|---|---|---|---|
| ▪ Digestion Using $HNO_3$ and $H_2O_2$ [a] | | | | | | |
| LGC 6137 | Estuarine sediment | Sediment | 12.4±1.8 | 12.1±2.18 | 24 | 98 |
| LGC 6137 | Estuarine sediment | Suspended particle | 12.4±1.8 | 11.9±1.81 | 10 | 96 |
| TORT 2 | Lobster hepatopancreas | Fish and shellfish | 21.6±1.8 | 23.4±1.04 | 16 | 108 |
| DORM 1 | Dogfish mussel | Fish and shellfish | 17.7±2.1 | 16.4±2.8 | 12 | 93 |
| Kelp* | Seaweed | Plant | (44±1.1) | 47±3.6 | 6 | 107 |
| ▪ Extraction using enzymes [b] | | | | | | |
| TORT 2 | Lobster hepatopancreas | Fish and shellfish | 21.6±1.8 | 22.3±2.0 | 14 | 103 |
| DORM 1 | Dogfish mussel | Fish and shellfish | 17.7±2.1 | 16.9±1.6 | 6 | 95 |
| Kelp* | Seaweed | Plant | (44±1.1) | 40±3.8 | 4 | 91 |
| ▪ Extraction using phosphoric acid [c] | | | | | | |
| LGC 6137 | Estuarine sediment | Sediment | 12.4±1.8 | 12.6±1.4 | 12 | 102 |
| LGC 6137 | Estuarine sediment | SPM | 12.4±1.8 | 12.4±1.21 | 4 | 100 |

[a] Nitric acid digestion procedure is shown in Table 2-4; [b] Enzymatic extraction procedures are shown in Table 2-5 (trypsin) and Table 2-6 (cellulase); [c] Phosphoric acid extraction procedure for sediments is shown in Table 2-7; *Kelp (*Ascophyllum nodosum*) –in-house reference material, reference value for 'total' arsenic = 44±1.1 $\mu g\ g^{-1}$ dry weight[33].

Each real sample analysis used a CRM which would, where possible, match that sample type under investigation as part of the validation protocol. For example, the CRM LGC 6137 was used to validate any sediment sample analysis.

The results show good acid digestion and extraction efficiencies for all reference materials, covering the range 91-108%. The species extraction efficiencies using phosphoric acid and enzymes show 100-102% and 95-103%, respectively.

- **Detection limits and blank concentrations for 'total' arsenic in sample analysis**

To ensure accuracy and inter-analytical precision, detection limits (determined as 3 times the standard deviation of the blank) and blank concentrations obtained for total arsenic analyses in samples using ICP-MS detection are shown in Table 2-15.

**Table 2-15 Detection limits (3 x standard deviation) and blank concentrations determined during analyses for total arsenic in shellfish, seaweed and sediment, digested using $HNO_3$ and $H_2O_2$.**

| Sample type | Blank concentration ($\mu g\ g^{-1}$) | Detection limit (3 x SD) ($\mu g\ g^{-1}$) |
|---|---|---|
| **Tamar** | | |
| Shellfish | 0.03 | 0.15 |
| Seaweed | 0.07 | 0.12 |
| Sediment | 0.06 | 0.18 |
| **Pak Pa-Nang*** | | |
| Fish and shellfish | 0.02 | 0.03 |
| Rice plant | 0.04 | 0.03 |
| SPM | 0.04 | 0.06 |
| Sediment | 0.05 | 0.03 |

* Refer to the Pak Pa-Nang River Basin sampling study in Chapter 3 and the analytical results in Chapter 4.

**• Pilot study on Tamar samples for total arsenic and arsenic species**

Mussel, cockle, green seaweed, brown seaweed and sediment samples were collected for the determination of 'total' arsenic in the Tamar Estuary. The values for total arsenic in the samples are shown in Table 2-16. The concentrations of total arsenic in the Tamar Estuary, molluscs (13-17μg $g^{-1}$), brown seaweed (93±6μg $g^{-1}$), green seaweed (8.6±0.1μg $g^{-1}$) and sediments (67±2μg $g^{-1}$), where all concentrations are on a dry weight basis, are found to be relatively high compared with other areas (as shown by the arsenic concentrations in a variety of aquatic sediments in section 1.4.4 and aquatic biota in section 1.5.5, Chapter 1). The Tamar River Basin is in SW England and during the mid 19$^{th}$ century, this region was one of the world's largest producers of tin, copper and arsenic[36]. Arsenopyrite was the only economically extractable form of arsenic and generally, these lodes were found in east-west trends. Arsenic ore roasting and oxide vapour condensing, which can give a pure arsenic compound up to 99%, used to be processed on various sites of the Tamar Basin[37]. Arsenic contamination in the Tamar Estuary is attributable to mobilization from the old mines, ore roasters and smelting sites. Hence, giving rise to the elevated of arsenic seen in the Tamar samples in this pilot study.

For arsenic species in the samples, the analytical results show good extraction efficiencies, in the range 88-108% of arsenic compared with the total arsenic from a nitric acid digestion. AsB (13-17 μg $g^{-1}$) was the major species found in mussel and cockle tissues with a small amount (0.5-0.6 μg $g^{-1}$) of inorganic arsenic. MMA (0.14 μg $g^{-1}$) was also found in trace amounts in mussels. Arsenic in sediments only revealed the inorganic species, $As^{V}$ and $As^{III}$, comprising 93 and 7%, respectively of the total arsenic content. The arsenic species compositions in the Tamar mussel, cockle and sediment samples compare well with the literature values (see the conclusion of arsenic species in different marine compartment in Table 1-9, Chapter 1). The results for the green and brown seaweed were not confirmed for AsB and MMA. The chromatograms, when studied were not perfectly matched in retention times to the standard chromatograms (Figure 2-8). Those species were shown to be absent using the spiking technique stated earlier. After the green seaweed and brown seaweed extracts were spiked with AsB and MMA and re-injected to the HPLC-ICP-MS system, the results revealed that a compound with similar retention characteristics to MMA constituted the major species in both samples. A compound with similar retention characteristics to AsB was also

observed but at lower concentrations. It is known from the literature that the major arsenic species found in seaweeds are the arsenosugars[38-44] (see section 1.4.5 and Figure 1.4, Chapter 1).

**Table 2-16 Results of analyses for arsenic concentration in the samples (dry weight) from the Tamar Estuary; all values are given in μg $g^{-1}$ of As, mean ± standard deviation (n=3).**

| Sample | Total As | AsB | MMA | Inorganic As | Total As in extract | Extraction efficiency (%) |
|---|---|---|---|---|---|---|
| Mussel | 13±1.2 | 14.3±0.2 | 0.14 | 0.5±0.03 | 14±1.1 | 108 |
| Cockle | 17±1.6 | 14.1±0.2 | ND | 0.6±0.03 | 15±1.8 | 88 |
| Green seaweed | 8.6±0.1 | [1.2±0.2] | [3.6±0.3] | 2.8±0.1 | 8.4±1.6 | 98 |
| Brown seaweed | 93±6 | [15±3] | [56±6] | 8.6±3 | 88±14 | 95 |
| Sediment | 67±2 | NP | ND | 4.7±0.2($As^{III}$) 60±8.2($As^{V}$) | 63±3.4 | 94 |

DMA was not found in any sample; ND=Not detectable; NP=Not performed; [ ] Unknown species with retention times comparable to AsB or MMA.

The absence of available standards for these compounds results in these majors peaks being unidentified at present. The complex nature of the many different arsenosugars present in seaweed results in a number of similar, if not identical, retention times to those of the more common arsenic species when using this chromatographic system. As a result, even the inorganic arsenic peak identified by spiking may be called into question. This coincidence of peaks has been encountered previously and subsequent hydride generation investigations on the extracts demonstrated that little to no readily reducible arsenic was present in a number of seaweeds[33].

The recommended threshold value for arsenic in food in the UK is 1 μg $g^{-1}$ wet weight (Ministry of Agriculture, Fisheries and food, 1982)[45]. At present, this is independent of the species although this value is under revision with the intent to define it in terms of inorganic arsenic at 1 μg $g^{-1}$. It is the inorganic arsenic species, $As^{III}/As^{V}$ which are considered most harmful to human health[2,3]. In Table 2-16, all the concentrations of arsenic are based on dry weight of sample. The moisture contents in the Tamar samples are 64, 75, 91 and 83% for mussel, cockle, green seaweed and brown seaweed, respectively. The corrected concentrations for inorganic arsenic in the wet weight samples are shown in Table 2-17.

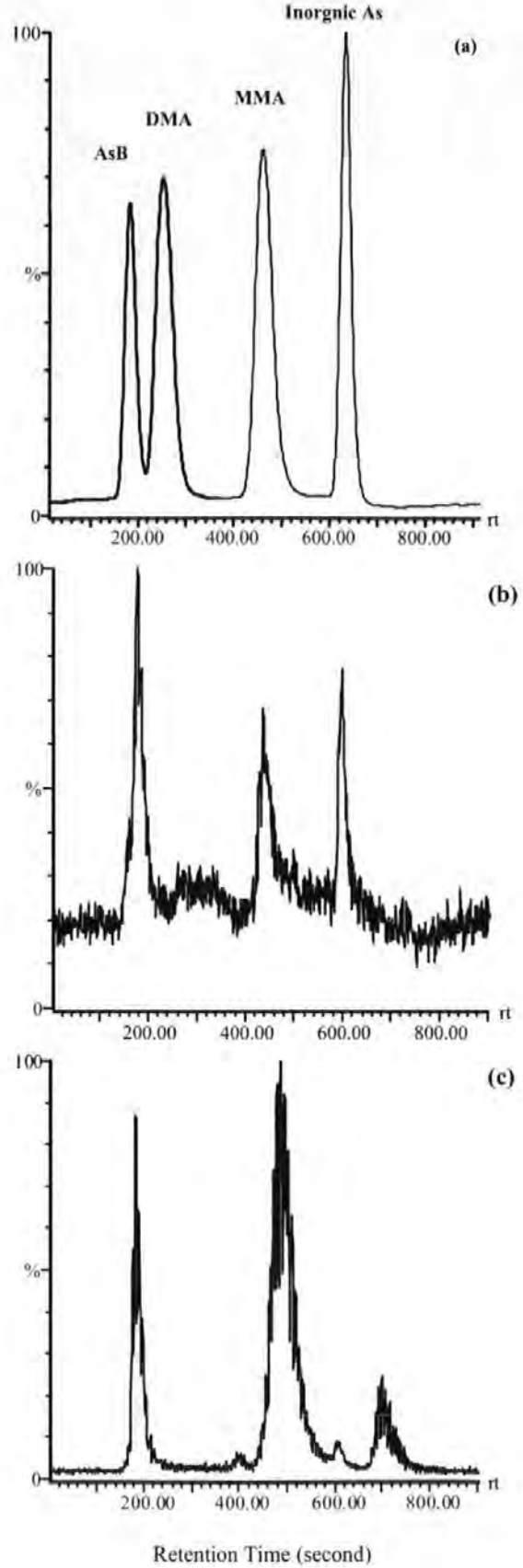


**Figure 2-8 Chromatograms of arsenic species; (a) standard AsB, DMA, MMA and inorganic As 200 ppb As in each species, (b) Green seaweed extract and (c) Brown seaweed extract (seaweed extraction procedure for arsenic species, using cellulase is shown in Table 2-6).**

**Table 2-17 Arsenic concentration in the samples from the Tamar Estuary (μg $g^{-1}$), mean ± standard deviation (n=3).**

| Sample | Inorganic As ( dry weight) | Moisture content (%) | Inorganic As ( wet weight) |
|---|---|---|---|
| Mussel | 0.5±0.03 | 64 | 0.18 |
| Cockle | 0.6±0.03 | 75 | 0.15 |
| Green seaweed | 2.8±0.1 | 91 | 0.25 |
| Brown seaweed | 8.6±3 | 83 | 1.5 |

The inorganic arsenic content in each sample (wet weight) is under the proposed threshold limit of 1 μg $g^{-1}$ for arsenic in food except for the brown seaweed which slightly exceeds the limit. Introduction of this material into the food chain, through its use in agricultural practices, if not directly ingested, may pose a risk to humans and other animals.

### 2.3.5 Mass balance calculation

A mass balance calculation (as previously defined in Figure 2-2) was performed using the analytical results from the Tamar pilot study. In Table 2-13, each of the arsenic species from each sample were summed. The total arsenic concentration from the acid digestion of each sample should be equal to both the enzyme extracted total arsenic concentration and the sum of 'arsenic from species'. The comparison of the mass balances for each sample is shown in the Table 2-18.

**Table 2-18 Arsenic mass balance for the Tamar samples; mean ± standard deviation (n=3).**

| Sample | Total arsenic [a] (μg $g^{-1}$) | Total arsenic in extract (μg $g^{-1}$) | Sum of arsenic from species (μg $g^{-1}$) |
|---|---|---|---|
| Mussel | 13±1.2 | 14±1.1 | 14.9 |
| Cockle | 17±1.6 | 15±1.8 | 14.9 |
| Green seaweed | 8.6±0.1 | 8.4±1.6 | 7.6 |
| Brown seaweed | 93±6 | 88±14 | 79.6 |
| Sediment | 67±2 | 63±3.4 | 64.7 |

[a] Total arsenic using the Nitric acid microwave digestion technique.

Inspection of the results indicates that, in general, there is good agreement for arsenic concentrations in the digests, in the extracts and the sum of arsenic from species for mussel, cockle and sediment samples. The only slight exception is the seaweed samples with the approximate 9.5% shortfall found from the sum of arsenic species compared with the concentration values in the extracts. As was noted, the chromatograms obtained from the green seaweed and the brown seaweed extracts can show an unresolved complex mixture of arsenic-containing species. Since identification of the species has, thus far, proved difficult, only a general estimate for the concentration of arsenic from species can be made. Hence the mass balance for seaweed does not tally for these samples. The mass balance for the Pak Pa-Nang samples will be considered in Chapter 4.

## 2.4 Summary of the methodology studies for total arsenic and arsenic species

Laboratory studies have been performed to meet the aims of developing and validating techniques for the determination of total arsenic and arsenic species in both reference materials and real samples. Sample digestion for 'total' arsenic was achieved using microwave-assisted Teflon bombs with concentrated $HNO_3$ acting as oxidizing agent and with the addition of $H_2O_2$ to increase the oxidizing power. Sample digestion time was optimized and near-to-full digestion efficiency was achieved using a three-minute heating period, a sample cool down step and then followed by two-minutes further heating to complete the digest. Sample extractions for arsenic species were successful, when based on enzymolysis for biota, using trypsin for fauna and cellulase for flora. Arsenic species in sediments were successfully extracted with a high efficiency (100-102%) using phosphoric acid extractant, and a focused microwave digester. ICP-MS was the instrument of choice to determine 'total' arsenic in digests and extracts with $N_2$-addition at 4% to the injector flow to suppress polyatomic interference. The major arsenic species were successfully separated using an anion-exchange resin-based HPLC system and four different eluents. When coupled with ICP-MS, using a short teflon tube, low limits of detection and suitable sensitivity was achieved for most species analyses.

Validation, using certified reference materials and mass balance calculations, was used to monitor and optimise the methodology and to ensure the quality of results maintained was high. This methodology has been shown to be ready to be applied to the target area

of the Pak Pa-Nang River Basin, which is an area of arsenic contamination and concern for human health.

It is to be noted that the methodology described in this chapter for the determination of dissolved 'total' and inorganic arsenic in water samples from the Pak Pa-Nang River Basin was performed at Chulalongkorn University, Thailand with the assistance of researchers from the Department of Marine Sciences.

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# Chapter 3

# Sampling, monitoring and hydrography of the Pak Pa-Nang River Basin

## 3.1 Introduction to the Pak Pa-Nang

This chapter gives a general description of the Pak Pa-Nang River Basin and the problems that are faced by the people of the region. It also describes the sampling in the rivers and the estuary and, together with the analyses, and that was undertaken in 2001, 2002 and 2003. The results of the analyses of the samples are shown in Chapter 3 and Chapter 4. There are no literature reports of the hydrography and water quality of the Pak Pa-Nang River Basin and Chapter 3 will describe the results of three surveys where these parameters were measured. These results will also provide a useful background to the main study on the arsenic distributions.

### 3.1.1 Socio-economic factors

The Pak Pa-Nang River Basin is subject to many conflicting human uses and influences[1]. The basin supports a population of 500,000 inhabitants, amongst which are rice farmers (paddy fields covering approximately 25% of the catchment area), orchard and rubber tree growers, fishermen and shrimp farmers. Three decades ago the area was prosperous and because of soil fertility rice exports contributed significantly to the local economy. Since 1970 the region has become one of the poorest communities in southern Thailand due to population growth, a failure in agricultural practices and devastation of the natural forest in the upper catchment. In the late 1980's shrimp farming was introduced into the area, giving very high incomes but this caused severe

conflicts over water use. Apart from the major Pak Pa-Nang River the region also has three other rivers (often called canals), i.e. the Bang Chak, the Pak Nakorn and the Pak Phaya. They all have their water supply from the mountainous region in the upper catchment area (see Ronpiboon area in Figure 3-1) where many small streams combine to form these main canals.

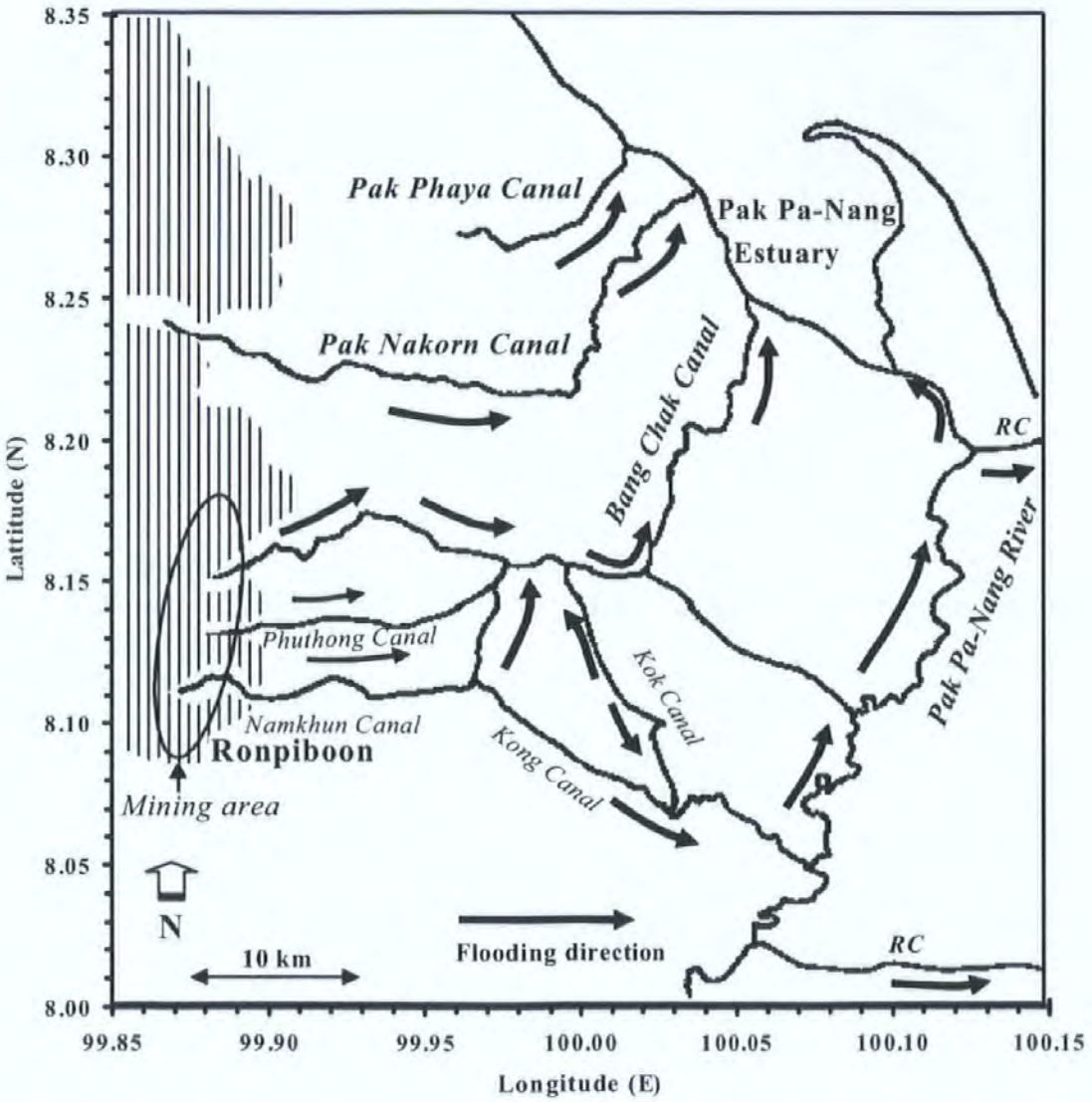


**Figure 3-1 Pak Pa-Nang River Basin, Nakorn Si Thammarat, Thailand. The figure shows the main directions of the water transport in the basin (source: Irrigation Department, Nakorn Si Thammarat); flood relief channels (*RC*) from the Pak Pa-Nang River to the Gulf of Thailand are shown on the figure.**

The degradation of the Pak Pa-Nang River Basin has also been of concern because its catchment is mineralized with substantial deposits of tin forming part of the SE Asian Tin Belt. The presence of arsenopyrite gives rise to high arsenic concentrations, mobilized during past mining activity and erosion from the abandoned tin mines. The

consequences have been poor water quality [2,3] and serious detrimental effects on human health [4,5] and on the environment[6-8]. Although there have been attempts to reduce the impact of the arsenic contamination[9], by improving the quality of drinking water, the number of cases of cancer arising from arsenic poisoning appear still to be increasing [10].

### 3.1.2 Water management

The Pak Pa-Nang River Basin is a fertile coastal plain in southern Thailand with a total area of 300 kHa and water is a very important resource. The Pak Pa-Nang River (Figure 3-1) is 110 km long with a catchment area of about 1,000 $km^2$. River flows vary between 10 $m^3 s^{-1}$ in the dry season (March to September) to 120 $m^3 s^{-1}$ in the period of the north east monsoon (November to January)[11]. The climate of the Pak Pa-Nang region is tropical monsoon with an annual average precipitation of about 2,000 mm. During southwest monsoon from May to October, the wind direction is predominantly from the southwest and the direction changes to northeast during the northeast monsoon from November to January.

Annual rainfall data are shown in Figure 3-2, where the large differences between the wet and dry seasons can be seen. During this study the sampling was carried out under both dry and wet conditions. The dry season sampling campaign was conducted in August 2001, when the accumulated rainfall in that month was 124 mm. The first wet season sampling campaign was conducted in December 2002 with an accumulated rainfall of 260 mm for the month. An even higher accumulation of rainfall amounting to 588 mm was recorded in December 2003, when the second wet season sampling campaign was undertaken.

Because of the extremes of rainfall, the availability of water varies considerably between the dry and wet seasons and water management has to be done carefully. The main issues for water managers (in this case the Irrigation Department in Nakorn Si Thammarat) are threefold:- (I) maintaining the availability of fresh water for rice farmers and to prevent seawater incursions, (II) the prevention of flooding into villages during the NE monsoon and (III) the prevention of freshwater encroachment into shrimp farming areas, which are typically dependent on saline water. In addition, the saline water discharged from shrimp farms, often in an uncontrolled way, may interfere with rice growing. Thus, in more recent times a complex water management system has

been developed, including newly dug or re-routed canals, e.g. the Kok Canal (Figure 3-1). The general directions of the water flows in the basin are shown in Figure 3-1, although there may be changes in flow direction by the management. At various points on the rivers and canals there are gates, some are manual and some are automatic, which allow the managers to re-direct the water as required, during severe flood events and for farming purposes. High flow conditions can develop rapidly at any time in the wet season, for example, in 2003, 192 mm of rain fell in less than 24 hours causing massive flooding in the region, the impact of which may have been reduced by the flood relief channels. For example, there are two flood relief channels from the Pak Pa-Nang to the Gulf of Thailand, the newest channel is shown at the bottom right of Figure 3-1 and the one near the estuary is shown in Figure 3-3.

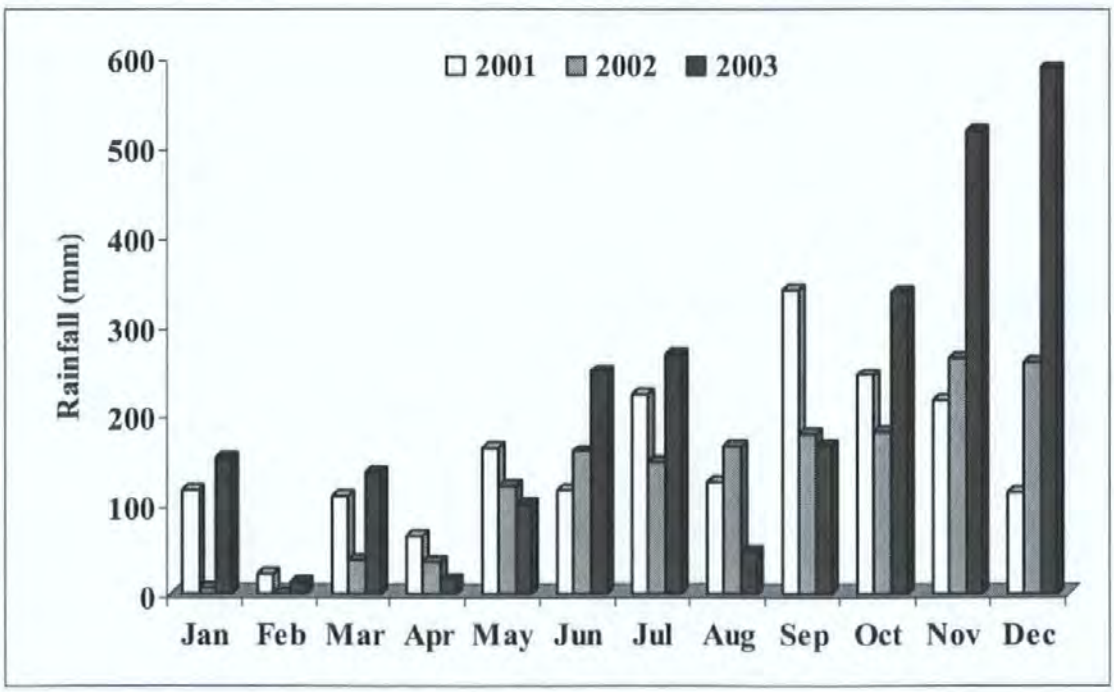


**Figure 3-2 Monthly rainfall of the Pak Pa-Nang River Basin, during 2001, 2002 and 2003 (Source of data: Meteorological Department, Ministry of Lands and Agriculture, Thailand).**

The complexity of the waterways throughout the region is illustrated in Figure 3-3 for the eastern side of the estuary. The thick blue line represents the path of the Pak Pa-Nang River. It has the largest gate, with automatic barriers, and is shown as the orange triangle at the centre of the river. Also visible as thin blue lines are the many small streams and canals used to supply water to individual fields and to carry away waste


**Figure 3-3** **Aerial map of the eastern side of Pak Pa-Nang River Basin; (Source: Ministry of Natural Resources and Environment, Thailand).**

water from small settlements. A recent development is a new man-made relief channel going from the right-hand loop of the Pak Pa-Nang to the Gulf of Thailand. This was an attempt to prevent flooding in the town of Pak Pa-Nang near the river mouth (the brown area of the Figure 3-3). The small squares scattered all along the coast are sites of shrimp farms and the houses of the shrimp farmers, both abandoned and in current use. The water management is not perfect at the moment, especially the operation of the Pak Pa-Nang barrier (Figure 3-4), which controls both freshwater flows to the estuary and seawater intrusions into the river. During the three surveys, we spoke to many local inhabitants who gave personal evidence that the barrier appeared to change the condition of the water. When the barrier was fully closed (to supply freshwater to the rice farmers and to prevent the seawater intrusion) those living by the river experienced a "bad smell" coming from the water, possibly as a result of the biochemical oxygen demand. Other inhabitants were worried that the barrier may have caused a growth in the water hyacinth in the freshwater behind the dam, that small fish had disappeared from the estuary and that no account had been taken of the need to consider pollution transport from the catchment (not covered by Figure 3-3). In 2002, the Irrigation Department, the managers of the barrier, partially opened the barrier, to allow free flow, in an attempt to stop further criticism from the local people.

**Figure 3-4 The Pak Pa-Nang River Barrier (opened in September 1999).**

The waters from the Pak Pa-Nang River Basin drain into Pak Pa-Nang Estuary (see Fig. 3-3). The estuary has dimensions of 15 km x 20 km, with water depths in the range 0.5 to 12 m. The eastern shore is dominated by mangrove forest. The tides in the estuary are semi-diurnal with a maximum range of no more than 1 m. The flux of riverine sediments to the estuary has been estimated to be 1.2 Mt $a^{-1}$, with approximately 70% being delivered during the north east monsoon. The annual deposition of sediment in the near shore is 640 kt and another 350 kt are deposited further out in the estuary[1].

## 3.2 Sampling procedure

### 3.2.1 Pak Pa-Nang River Basin sampling

The main objectives of the sampling in the river basin were as follows:

1) to obtain water, suspended particulate matter (SPM) and sediments over a wide geographic coverage of canals and rivers and of the arsenic contaminated area, so that determinations of arsenic concentrations and arsenic speciation could be made.

2) to make measurements of water quality (conductivity/salinity; temperature; SPM concentration; nutrients; chlorophyll), so that the water quality in the river and in the canals can be described.

3) to collect biological samples from a paddy field and a shrimp farm, so that determinations of arsenic concentrations and arsenic speciation could be made.

The primary sampling was in the main stem of the Pak Pa-Nang and several of the major canals draining to the estuary (i.e., Bang Chak Canal, Pak Nakorn Canal and Pak Phaya Canal). During December 2002 the Pak Pa-Nang River, Bang Chak Canal, Pak Nakorn Canal and Pak Phaya Canal were all sampled. By sampling in sequence, we tried to cover a representative range of water types from the rivers to the very low salinity region of the estuary. However, it was not always possible to obtain each representative water sample because of difficulties with access to the various rivers, as a result of the area being underdeveloped. However, it was possible to obtain a reasonably comprehensive set of samples, including contaminated water, sediments and SPM from the mine area and the estuary. During the surveys and sediment and water sample collections, water quality parameters measured in each station. In 2003, we took some

repeat samples at the same sites as 2002 to verify the levels of arsenic obtained previously. In view of the size of the river basin area, it was decided that the optimum number of sediment cores was limited to seven stations. Sediment cores were collected at selected points along the various rivers (i.e. A1; A3; A3A; B3; B4; C1; C3; see Figure 3-5, see also Figure 4-2 for clarify) so that an assessment of arsenic deposition over time could be made.

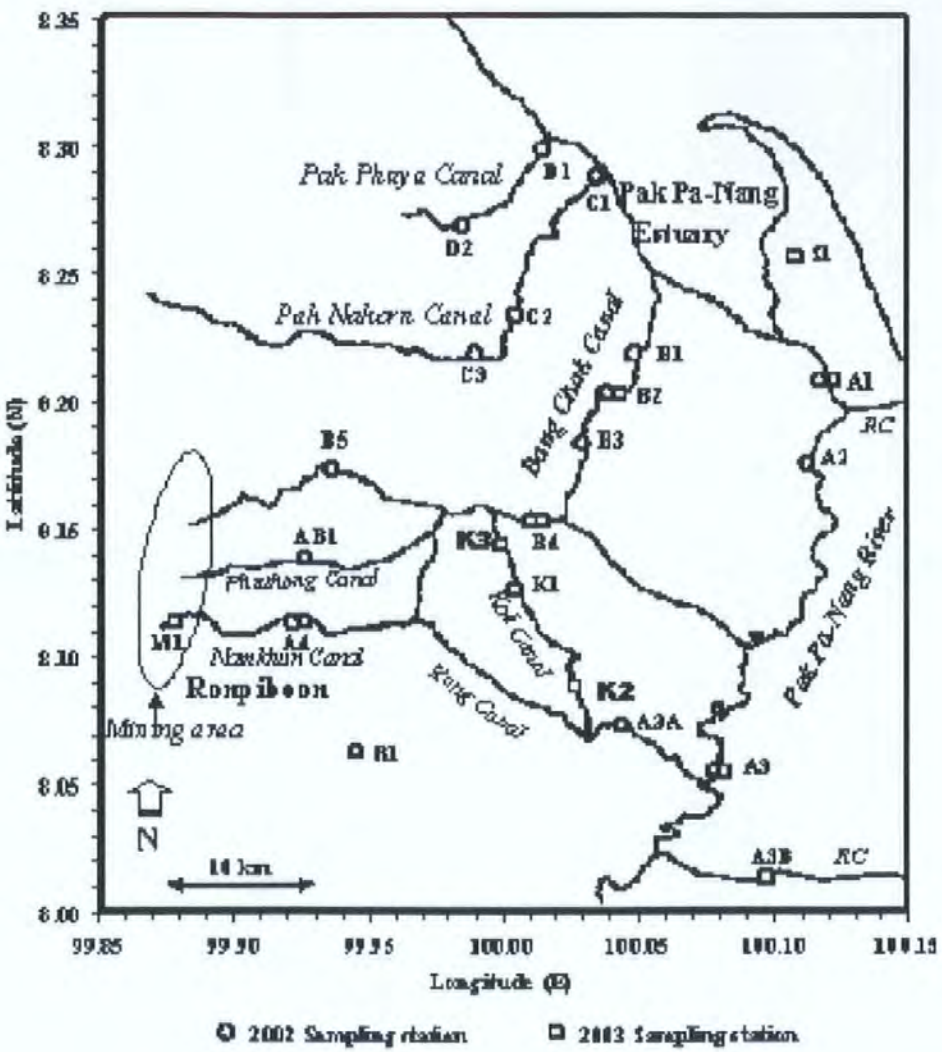


**Figure 3-5 Pak Pa-Nang River Basin sampling stations, 2002 and 2003; M1 –mining area station; R1 –rice field station, S1 –shrimp farm station, RC –flood relief channal.**

In 2003, it was decided extend the biological sampling into a paddy field and shrimp farm (Figure 3-5). At a shrimp farm along the coast, surface sediment and a water sample were collected, along with shrimps from the pond.

The selection of an appropriate paddy was more difficult because of the water management system it was not clear which paddy fields received water from the contaminated area. In this study, an area close to arsenic-affected area was selected for a characterization of irrigation water, soil and rice produced in the irrigated soils. The sampling was eventually located in Kourn Pung, a sub-district of the Ronpiboon district (see Figure 3-5 station R1), where an entire rice plant (including roots) was collected from the paddy field. The rice plant was in a mature state, approximately one metre high and was approaching the state when the seeds are produced. A sediment sample was collected from the area colonized by this rice, and a sample of rice grains produced in this area were also obtained from a rice mill.

### 3.2.2 Pak Pa-Nang Estuary sampling strategy

Sampling stations in the Pak Pa-Nang Estuary for dry season, 2001 and wet season, 2002 and 2003 surveys are shown in Figure 3-6. The stations were chosen to accomplish the objectives as follows:

- **Spatial coverage:**

1) to study the influence of river plumes draining to the Pak Pa-Nang Estuary, stations ppn1 to ppn4 were located in the river mouths of the Pak Pa-Nang River, Bang Chak Canal, Pak Nakorn River and Pak Phaya River, respectively.

2) to study the area of aquaculture in the estuary, stations ppn5 to ppn8 were located in the central area of the Pak Pa-Nang Estuary.

3) to obtain background data in the Gulf of Thailand, near the Pak Pa-Nang Estuary, station ppn9 was identified as being accessible either from the sea or from the shore.

4) to obtain more detail of the water quality parameters, intermediate stations, A-G (Figure 3-6) to add more data to the normal stations. Greater detail of the distribution of water quality parameters was obtained in 2003 when the survey were done in cooperation the Pollution Control Department (PCD), Ministry of Natural Resources and Environment, Thailand (Figure 3-7).

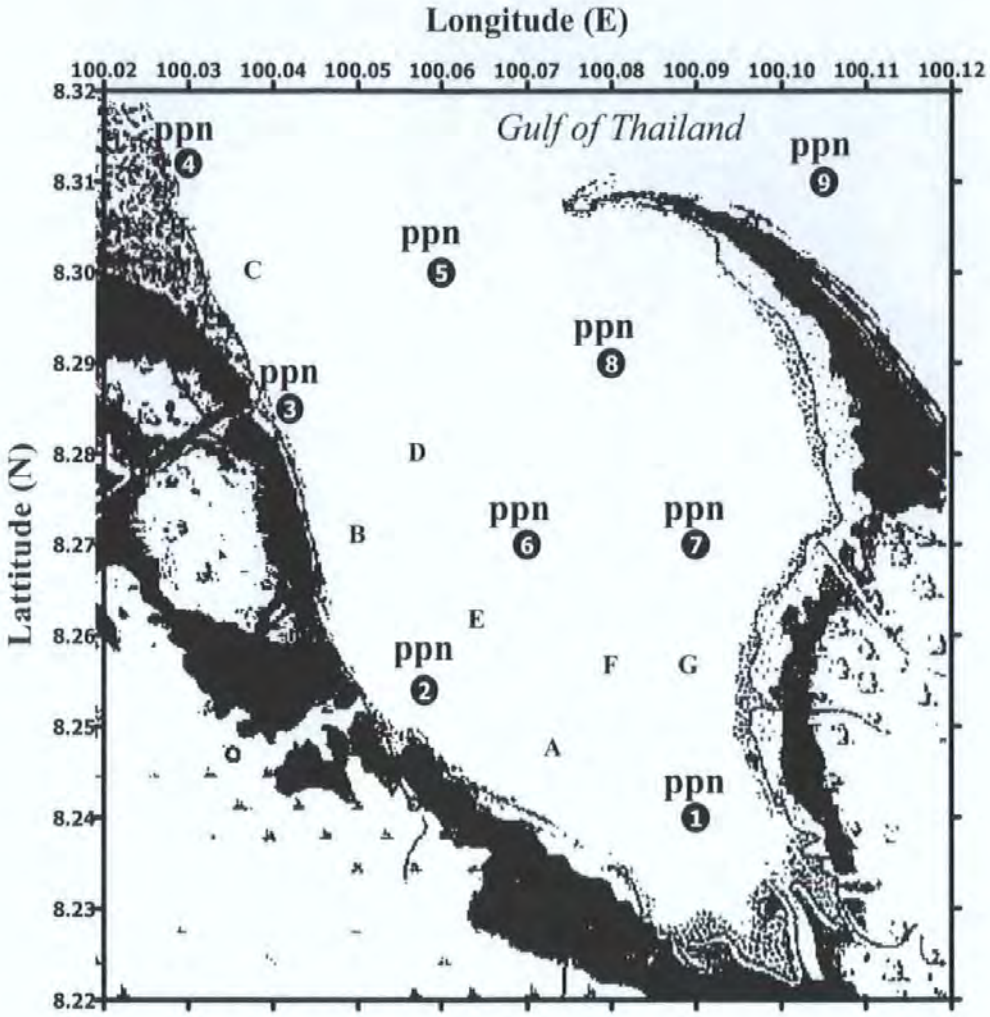


**Figure 3-6 Pak Pa-Nang Estuary sampling stations for surveys in 2001, 2002 and 2003. Main sampling stations ppn 1-9 (filled circles); intermediate stations A-G, where some physical parameters were measured to obtain more details of the distributions of water quality parameters and arsenic.**

- **Time-dependent coverage:**

5) to study the vertical distribution of metals in sediments, core samplings were carried out at four stations, ppn1, ppn2, ppn3, ppn4 in the 2002 survey and at station 35 in the 2003 survey.

6) to compare the seasonal effects in the estuary the stations the surveys were conducted in the dry season August 2001 and the wet season December 2002 and

December 2003. Also, to carry out surveys before and after heavy rain, if possible, so that the effects of the mobilization of arsenic could be studied.

7) to carry out water sampling during a tidal cycle at a station near the mouth of the estuary, i.e. ppn12 (Figure 3-6), in order that the transport of arsenic from the estuary to the Gulf of Thailand may be assessed.

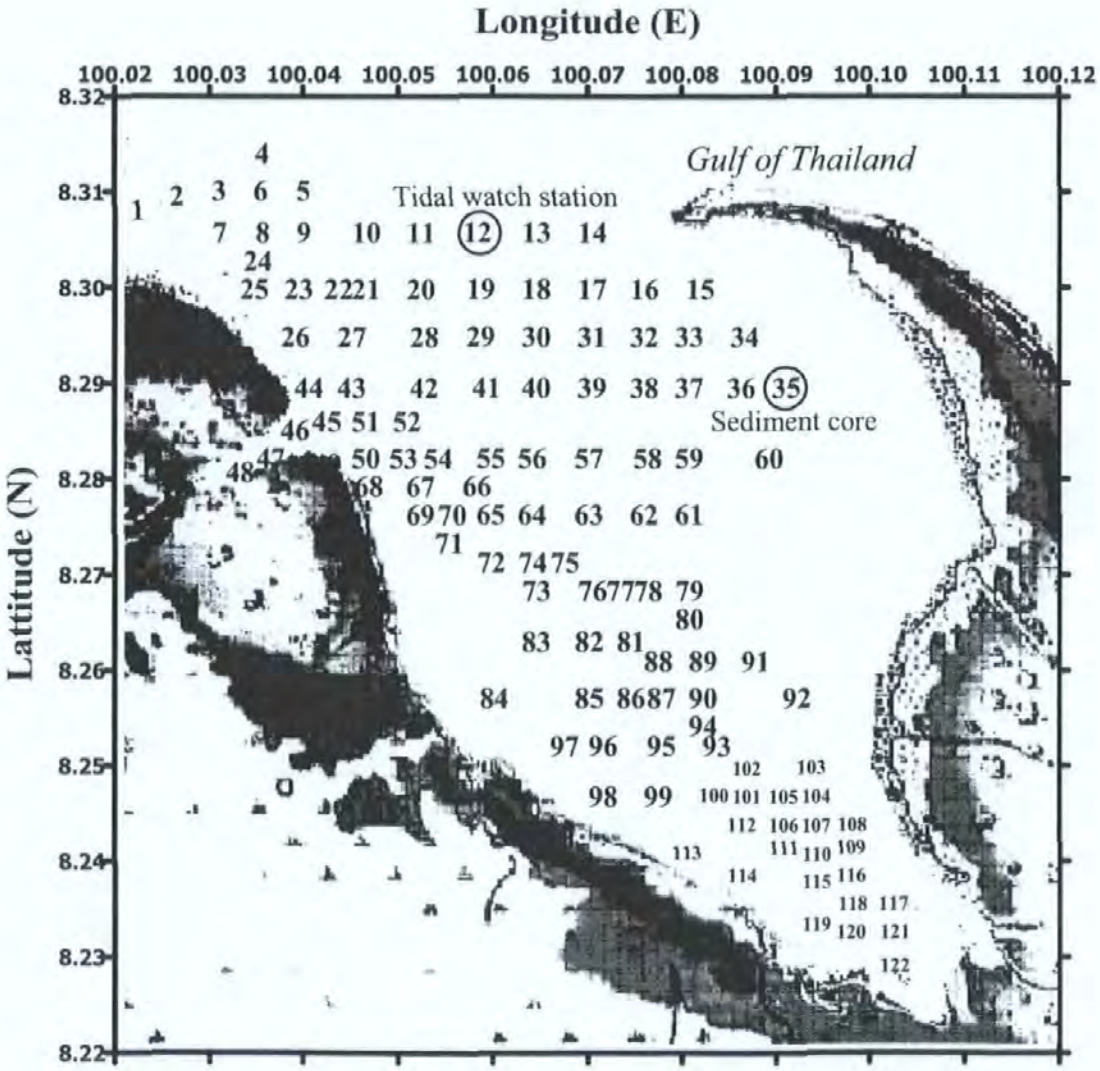


**Figure 3-7 Pak Pa-Nang Estuary monitoring and sampling stations for December 2003, conducted with the Pollution Control Department of the Ministry of Natural Resources and Environment. Station 35 is where a sediment core was collected; station 12 is where the tidal watch station was set up and water samples taken.**

### 3.2.3 In situ monitoring and sample collection

- **Vessels, positioning and *in situ* monitoring**

The first survey, in August 2001, was conducted using an inflatable boat fitted with an outboard. The survey team had a lack of local knowledge at this time and they were not prepared for the distances involved and the slow progress. Given these experiences, the 2002 and 2003 surveys in the estuary were carried out aboard a fast, long-tailed boat that could cover the area very quickly and could accommodate 4 scientists and 2 crew. The other advantage was that the skipper was a fisherman and he knew the local fishermen so it was possible to buy freshly caught seafood which was required for arsenic analysis. In 2003, sampling was aimed to operate at a smaller scale (122 stations) to obtain more details of the master variables in the estuary and this was done in cooperation with Pollution Control Department (PCD) of the Ministry of Natural Resources and Environment who supplied two more boats for sampling the stations shown in Figure 3-7.

Since one objective of the survey was to map the distribution of the concentrations of various parameters, it was necessary to locate the sampling stations accurately with a global positioning system (GPS). Once the vessel was positioned at the correct location the measurements were made, the data recorded and any sample (water, sediment or biota) was obtained and recorded. In 2003, three GPS systems were used and they were all checked for consistent readings before the start of the survey.

The *in situ* measurements were made from the vessel using calibrated sensors for (a) salinity and conductivity (using standard seawater); (b) temperature; (c) pH (using standard pH solution at 4 and 7) and (d) dissolved oxygen saturation (setting the 0% with a sodium sulphite solution and 100% in air). In the case of the joint survey in 2003 three separate sets of instruments were intensively, inter-calibrated in the laboratory prior to the survey by the whole team.

- **Tides**

The surveys were planned to be conducted about 2 hours either side of high water, assuming this would give a relatively stable distribution of water masses. However, it

was not always possible to organize the logistics and people, such that they agreed with that particular tidal condition. Thus, the monitoring and water and sediment sampling in 2001 occurred during a falling tide (Figure 3-8a) and the launch site was located north of ppn4, giving a long transit time to the first site. Also, the lack of water meant that the vessel grounded on several occasions and more time was lost. However, all the samples were obtained.

The tidal conditions were more favorable in 2002 and 2003, when high water was in the afternoon (Figure 3-8b and 3-8c, respectively). This allowed measurements to be made in shallow waters in the near shore regions and at approximately the same tidal state. Two separate surveys were made of the estuary in 2002, one where samples were collected and in situ monitoring made before any major storm and the other, after heavy rain, when in situ monitoring and sampling water quality parameters took place. There was more intensive sampling in 2003 with three vessels each covering a smaller area of the estuary and measurements being made at the surface and bottom of the water column. In December 2003, the PCD team also arranged a tidal watch near station 12 (Figure 3-7). The main intention was to measure physical parameters, such as current speed and direction, temperature and salinity. However, the PCD also collected water samples for this study every 4 hours for 24 hours, together with the respective values for salinity and temperature.

During the surveys water samples for arsenic analysis were collected at selected sites in one litre acid washed low density polyethylene bottles, which had been previously used for trace metal analysis. Samples for nutrient analysis and for particulate carbon were collected in separate acid washed one litre plastic bottles.

Samples of the surface sediments were obtained using a hand-held van Veen grab and samples oxic of sediments were placed immediately into acid-washed plastic bags. Sediment cores were collected using a gravity corer, with a replaceable, plastic liner. On retrieval the plastic liners were released, sealed at both ends, and stored in an ice-filled the cool box for transport to the laboratory.

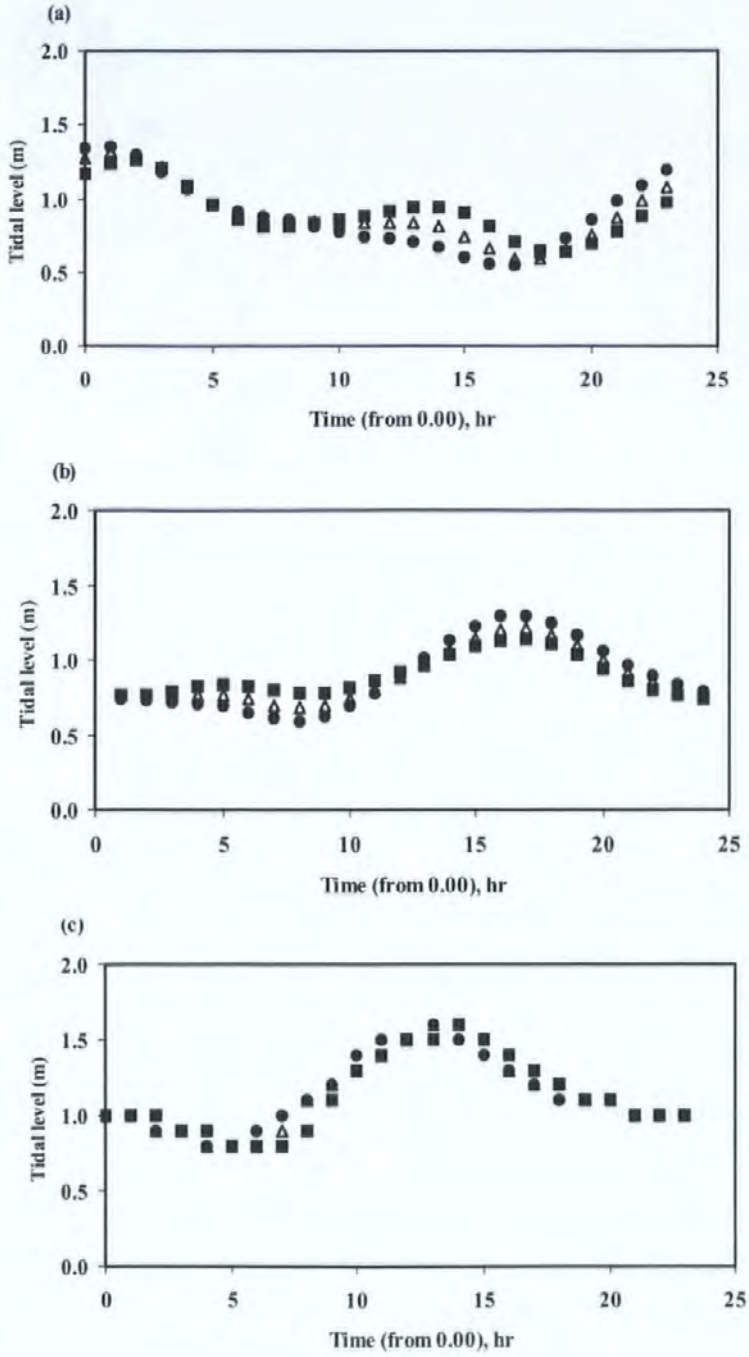


**Figure 3-8** **Tidal level (units are in meters above Lowest Astronomical Tide) in the Pak Pa-Nang area (Pak Phun Hydrographic Station 8° 32' N 100° 0' E); (a) dry season 2001, (b) wet season 2002 and (c) wet season 2003; ● -one day before sampling, Δ -sampling day, ■ -one day after sampling.**

### 3.2.4 Sample pre-treatment

- **Water samples**

Following collection the water samples were returned to the laboratory where they were filtered immediately using either pre-weighed, acid washed Millipore filters, with 0.45 μm pore size, or pre-weighed glass fiber filters, as appropriate. The volume of the filtrates was measured and acidified using concentrated HCl (i.e. 0.25 ml to 250 ml of water sample), then stored in a fridge prior to analysis for dissolved arsenic. The solids deposited on the filters were dried to constant weight and, together, with the volume of the filtrate were used to estimate the suspended particulate matter (SPM) concentration. The solid masses were also used to determine the concentrations of particulate arsenic.

- **Sediment samples**

In the laboratory of Walailak University (Nakorn Si Thammarat), surface sediment samples were pre-frozen for 24 hours in a freezer, held at -40°C, and were then rapidly transferred to a freeze drier for 24-48 hours until dry. The dried samples were packed with a vacuum packer and brought back to UK for analysis. Core sediment samples were sectioned into 2 cm slices using a nonmetallic cutter. The sediment in each section was pre-frozen, freeze-dried and vacuum packed in the same way as the surface sediment samples.

- **Fish and shellfish**

Fish and shellfish sample tissue preparation methods to achieve total arsenic and arsenic speciation have been done carefully. After obtaining the samples from the fishermen, they were stored in ice and quickly brought back to the laboratory at Walailak. With the exception of samples obtained in 2001, the muscle tissues were separated from bones and shells using only non-corrosive stainless steel instruments. Wherever possible the separations involved fish and shellfish of approximately equal size. The muscle tissues were rinsed thoroughly with deionised water then placed overnight in the ultra-low temperature freezer before being transferred to the freeze drier. Crab muscle tissue was removed from the claws, legs, and body after being freeze-dried. When removing muscle tissue from the crabs, care was taken not to include hepatopancreas tissue or

other organs. The freeze-dried samples were packed with a vacuum packer and brought back to UK for analysis, examples are shown in Figure 3-9.

In Plymouth, the freeze dried sediments, rice plant, fish and shellfish were ground using an agate mortar and pestle (the grinding was performed in a laminar flow clean hood). After the homogenizing the samples were sieved through a sieve with a mesh size of 180 μm and stored in small brown bottles and place in a dry place prior to analysis.

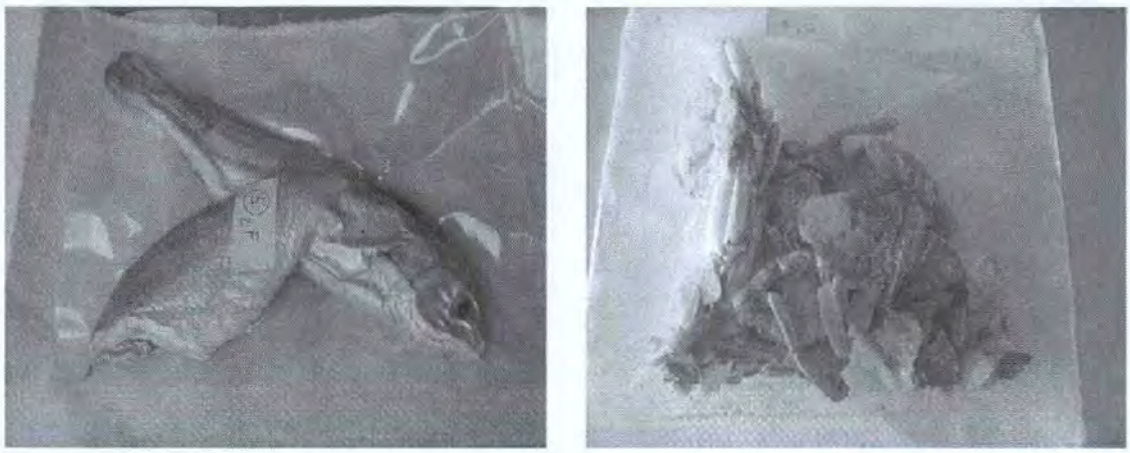

**Figure 3-9 Vacuum packs of freeze dried fish, Croaker (*Johnius belangerii*), left and a freeze dried crab, Swimming crab (*Portunus pelagicus*), right.**

- **Rice**

Before analysis, the rice plant sample was divided into three parts: (1) root, (2) stem and (3) leaf. The sediment, rice root, rice stem, rice leaf and rice grain samples were pre-frozen and the dried using a freeze dryer. The dried samples were vacuum-packed and brought back to the UK for total arsenic and arsenic species analysis.

## 3.3 Determinations of water quality parameters

The water quality parameters (i.e. nutrients and chlorophyll) and dissolved arsenic analysis were determined as part of the collaborative project with Chulalongkorn University, Bangkok. The methods for dissolved arsenic analysis are given previously in Chapter 2. The nutrient and chlorophyll samples were prepared and analysed immediately in the laboratory at Walailak University using the methods below.

### 3.3.1 Nutrient analyses

Dissolved nutrients were determined spectrophotometrically following the development of a coloured complex and were calibrated with standard solutions in waters of appropriate salinity to overcome the changes of refractive index[12]. The sum of the concentrations for nitrate and nitrite were determined by reduction to nitrite with copperized cadmium and derivatization by N-naphthylethylenediamine dihydrochloride and sulphanilamide to a pink dye, with photometric detection at 542 nm. The relative standard deviation of the analyses was 0.2% (n=5). Concentrations of orthophosphate were determined using the molybdenum blue reaction with detection at 660 nm and a relative standard deviation of 2%. For silicate determinations, the sample is acidified, prior to the addition of ammonium molybdate. The molybdosilicic acid formed is reduced with ascorbic acid, to yield a blue complex which is determined at 810 nm. Oxalic acid is added to avoid phosphate interference. Replicate analyses gave a relative standard deviation of 3%.

### 3.3.2 Chlorophyll analyses

The filtration of approximately 500 ml of water sample was carried out using a GF/F filter. Subsequently, the filter was extracted in a test tube with 10 ml of acetone in complete darkness for 20 hrs. The volume was made up to 12 ml with acetone and the absorbance determined at 750, 664, 630, 510 and 480 nm. The chlorophyll concentration was estimated from the algorithm following correction for the turbidity.

### 3.3.3 Determinations of particulate carbon and nitrogen

The freeze-dried sediments and GF/F filters containing deposited SPM were returned to Plymouth University for analysis. All analyses were carried out in triplicate on freeze-dried sediments and air-dried SPM on GF/F filters. Total carbon and nitrogen were determined combustiometrically using a Carlo Erba EA1110 elemental analyzer calibrated with acetanilide and cyclohexane standards. Particulate organic carbon and nitrogen were determined similarly on some samples following digestion of aliquots of the freeze-dried sediments in 1M HCl for 6 hrs. The accuracy of the method was confirmed using the BCSS-1 certified reference sediment, where the measured value for total carbon was 2.22 ± 0.03% (n=3) compared to the certified value of 2.19 ± 0.09 %

## 3.4 Results

### 3.4.1 Hydrography

Since it has the largest flow, the Pak Pa-Nang River exerts the greatest influence on the temperature-salinity (T-S) characteristics of the estuary. Figure 3-10 shows surface water salinity of the Pak Pa-Nang Estuary at about high water. The freshwater influence is most obvious near to the major rivers and the range of salinity is from 0.4 to 29. It is also seen that there is a "tongue" of high salinity water penetrating the estuary from the Gulf and it appears to be moving along the main deep water channel.

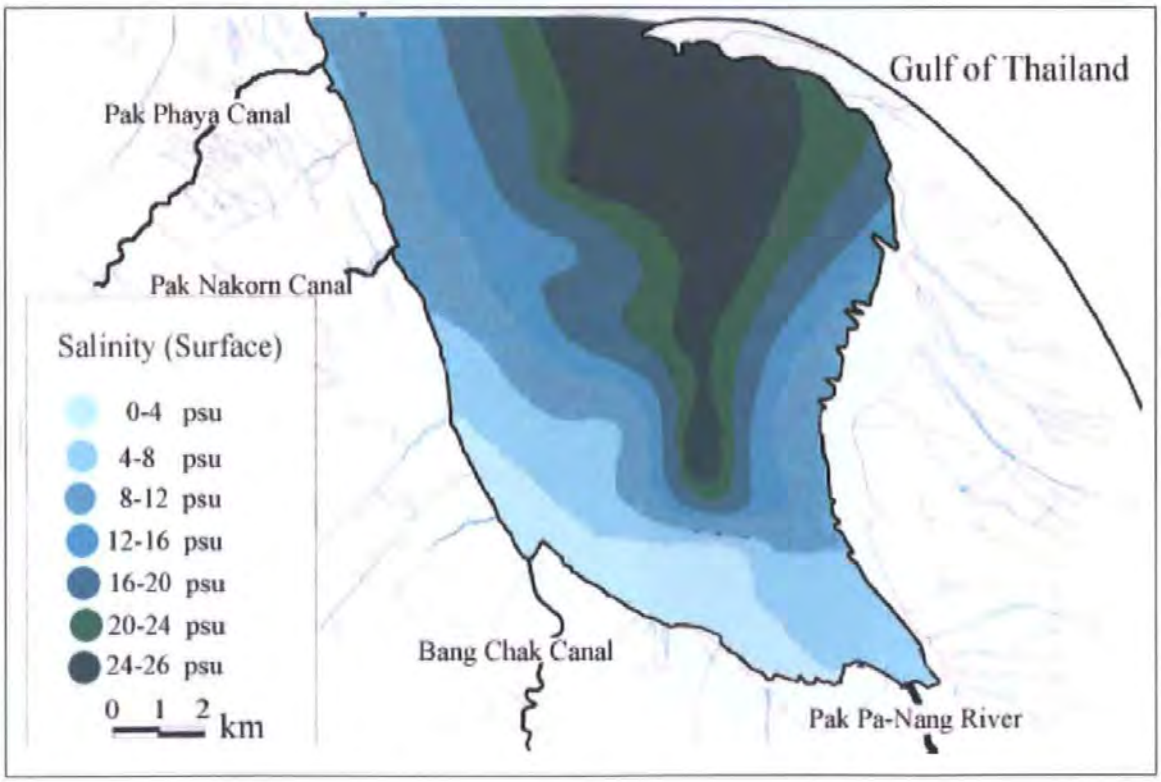


**Figure 3-10 Surface salinity profile of the Pak Pa-Nang Estuary during December, 2003 (Source: Pollution Control Department, Ministry of Natural Resources and Environment, Thailand).**

The T-S curves for each of the surveys of the Pak Pa-Nang are shown in Figure 3-11. For the dry season in August 2001, the T-S curve is ill-defined because high water was at 01.00 am (Figure 3-8a) and the survey had to be conducted around low water. The survey was therefore, several hours prior to high water when the waters were not in a relatively constant tidal state and the definition of water masses was less pronounced.

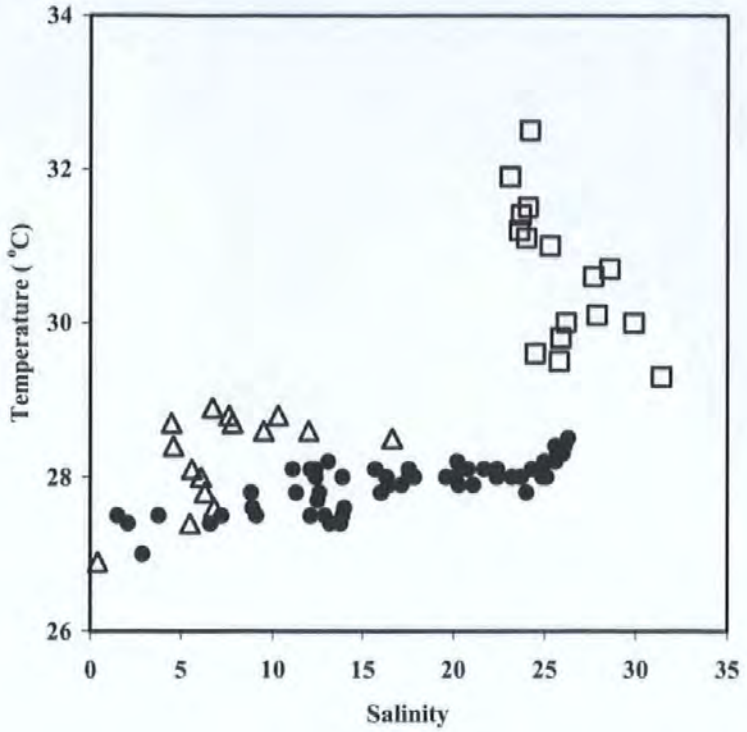


**Figure 3-11 Temperature-salinity characteristics of the Pak Pa-Nang Estuary; □ -August 2001; Δ -December 2002; • -December 2003.**

In general the salinities ranged from a low of 23.1 at station ppn7 to a high of 31.4 at the offshore station ppn9. The highest temperature was 32.5°C at station ppn2 and the lowest was 29.3°C at station ppn9. The relatively high water temperatures at stations within the estuary may have been increased by heat gained from sediments as the water covered them.

In 2002, an S-shaped T-S curve was obtained by conducting the survey rapidly around high water and immediately after heavy rainfall. The T-S curve clearly illustrates the influence of the largest freshwater inputs, namely the Pak Pa-Nang and the Bang Chak. Here the T-S curve is defined by the relatively warm waters of the Pak Pa-Nang River, with an effective zero salinity end member (EZSEM) value of 29°C, and the cooler waters of the Bang Chak, with an effective EZSEM of 27.5°C. Both EZSEMs were confirmed by measurements in the freshwater reaches of the respective rivers, i.e. for the Pak Pa-Nang 29.1°C at station A3 and for the Bang Chak 27.7°C at station B2. Thus, during flood conditions it may be possible to trace the sources of contaminants using the T-S characteristics of the waters in the estuary.

The T-S relationship for 2003, prior to any flooding, suggests that the waters of the estuary are mainly influenced by mixing of seawater from offshore with riverine water. Assuming linearity, the EZSEM of the T-S data gives a temperature of 27.3°C, which is similar to the mean value (27.8°C) determined in the freshwater reaches of the Pak Pa-Nang River. Thus, riverine water from the Pak Pa-Nang dominates the mixing processes in the estuary under normal flow conditions. During flood conditions the T-S characteristics of the estuary are modified such that fluvial inputs from other sources are detectable.

### 3.4.2 Water quality in the estuary

- **Suspended particulate matter**

Table 3-1 gives the SPM concentrations in the estuary during the three surveys. Generally the SPM concentrations are low, as shown by the mean values from 2001 and 2002 of 26±18 mg $l^{-1}$ and 31±19 mg $l^{-1}$, respectively. The 2002 was obtained before the rain event. These values indicate a weak riverine source of SPM and little sediment resuspension. The mean concentration of SPM found in 2003 was much higher, 245±198 mg $l^{-1}$, possibly as a result of high rainfall during wet season of 2003 (see charts of monthly rainfall in Figure 3-2). However, the winds appeared much stronger in 2003 and wave action could have resuspended sediments to create the higher concentration. The seasonal variation of SPM concentrations was suggested by Boromthanarat (1991) whose calculations indicated that the flux of riverine sediments to the estuary were approximately 1.2 Mt $a^{-1}$, with about 70% being delivered during the wet season[1].

**Table 3-1 SPM concentrations in the Pak Pa-Nang Estuary, mean ± standard deviation (dry weight).**

| Sampling | SPM concentration | |
|---|---|---|
| | Mean (mg $l^{-1}$) | Range (mg $l^{-1}$) |
| August, 2001 (n=9) | 26±18 | 2-64 |
| December, 2002 (n=8) | 31±19 | 9-61 |
| December, 2003 (n=12) | 245±198 | 27-245 |

- **Dissolved oxygen saturation**

The riverine waters, feeding into the estuary, had a mean dissolved oxygen saturation of 54%, possibly because of the biochemical oxygen demand (BOD) coming from domestic inputs from villages along their routes. BOD is a measure of the oxygen used by microorganisms to decompose organic matter such as dead plants, or sewage. In the estuary, the dissolved oxygen saturation was between 80 and 100%, for most of measurements and particularly in 2003 (Figure 3-12a), when the estuary was more turbulent because of weather conditions. In 2001, when the weather was calm, there was a significant oxygen decrease in the estuary, particularly in waters close to the river mouths. However, the water was supersaturated with oxygen at the offshore station ppn9, possibly due to phytoplankton growth. A similar situation existed in 2002 when the dissolved oxygen saturation ranged from 31% to 124%. The greatest oxygen depletion was again evident in low salinity waters. In 2003, there was less of an oxygen decrease with a mean of 92±6% in surface waters and 90±9% in the bottom waters. However, a group of samples (Figure 3-12a) whose values were relatively low and formed part of the plume of water associated with the input from the Pak Nakorn Canal, which passed through the major city of Nakorn Si Thammarat where it probably picked up domestic and industrial waste.

- **pH**

The mean pH of the riverine waters is 6.4, whereas the pH in the estuary generally falls within a narrow range between 7 and 8 (Figure 3-12b). In a few cases for each of the three surveys the pH exceeded 8 and this was generally when the dissolved oxygen saturation was greater than100%. This suggests that active photosynthesis, involving the removal $CO_2$, giving rise to increased pHs. In 2003 there were several cases where the pH was lower, at high salinity, than the general trend. The group of samples between salinities 10 and 20 were associated with the plume of the Pak Nakorn and the group of three at salinities greater than 25 were near to the eastern shore where discharges of seawater from shrimp farms are prevalent.

- **Particulate carbon**

The total carbon content of the suspended particles increases as the SPM concentration decreases (Figure 3-12c). The concentration of total carbon in riverine SPM had a

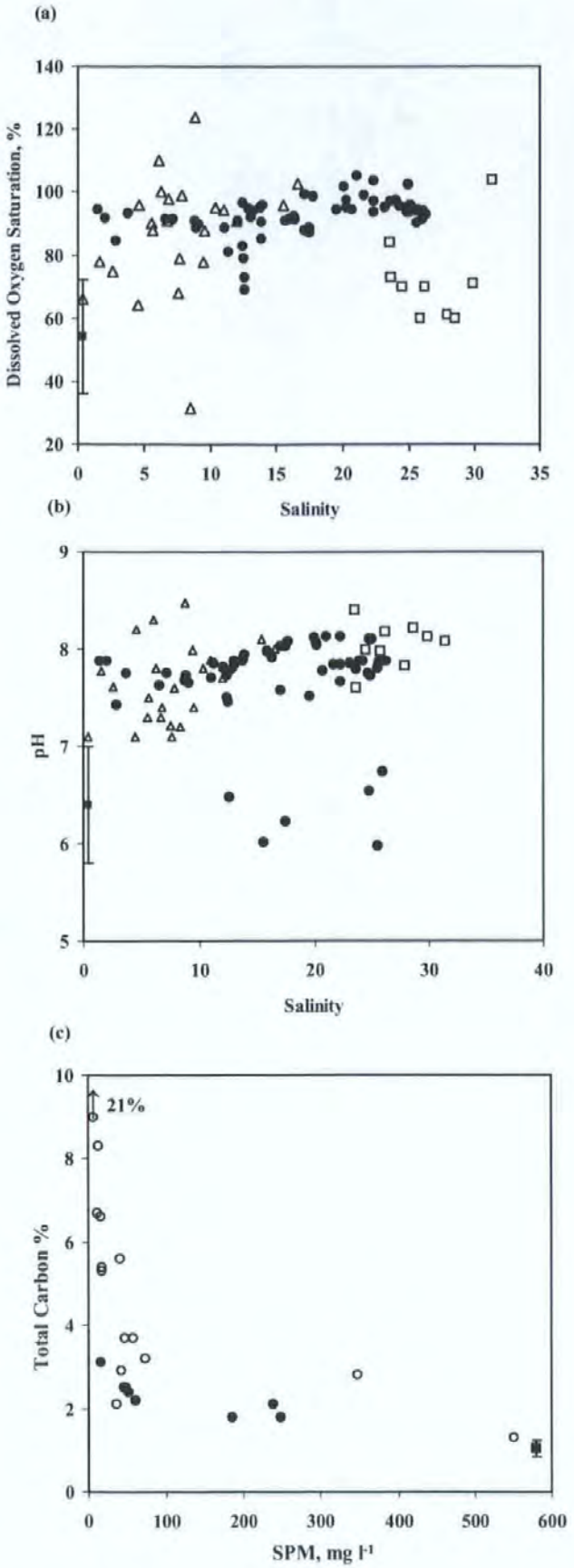


**Figure 3-12** Dissolved oxygen content, pH and particulate carbon in the Pak Pa-Nang Estuary and its rivers; (a) dissolved oxygen saturation versus salinity. □ -August 2001; Δ -December 2002; • -December 2003; (b) pH versus salinity. □ -August 2001; Δ -December 2002; • -December 2003; (c) total carbon versus concentration of suspended particulate matter • –estuarine SPM; ○ –riverine SPM; ■ –mean and standard deviation of the total carbon content of sediments.

maximum value of 21% at low SPM concentrations. In the estuary the maximum of total carbon is about 3% and its concentration declines as the SPM increases. The trend of decreasing concentration with increased SPM load has been observed for particulate metals in estuaries[13]. This happens because coarser material, with lower concentrations of a particulate constituent, can be remobilized from the sediments under turbulent conditions and acts to dilute particulate concentrations at higher turbidities. However, some of the carbon in SPM may be sewage-derived and could be undergoing mineralization during its passage to the sea, as has been shown for several European estuaries[14]. Thus, mineralisation may also contribute to the decrease carbon concentrations, as well as the oxygen deficiency referred to in Figure 3-12a.

Compared to the SPM, the concentration of total carbon in the sediments of the estuary is relatively low (Figure 3-12c). The mean concentration of total carbon was constant about 1% in both 2001 and 2002, while the concentration of total nitrogen declined from 0.28% in 2001 to 0.12% in 2002 (Table 3-2). Sediment samples from 2001 showed that about 61% of the total carbon was as organic carbon, while the organic nitrogen was about 25%. The sediments in the estuary had variable C:N ratios from about 3.5 in 2001 to 9 in 2002 (Figure 3-13a) but the sediments in the rivers had a relatively constant C:N ratio of 10.7 ± 2.6, possibly because the rivers are rapidly flushed (Figure 3-13b). The SPM in the rivers also had a high total carbon compared to the sediment, which suggest the presence of carbon from another source, such as domestic waste. However, it is not possible to define the source of the sedimentary carbon, since the C:N ratios cannot be used to establish the source of the particles because of biochemical modification by bacteria after deposition[15].

**Table 3-2 Concentrations of total carbon and organic carbon in the sediments of the Pak Pa-Nang Estuary and its rivers.**

| Sample | Concentrations (%) | |
|---|---|---|
| | Carbon | Nitrogen |
| **Estuary ~ 2001** | | |
| Total | 1.05 ± 0.21 | 0.28 ± 0.07 |
| Organic | 0.64 ± 0.23 | 0.07 ± 0.03 |
| **Estuary ~ 2002** | | |
| Total | 1.04 ± 0.19 | 0.12 ± 0.05 |
| **Rivers ~ 2002** | | |
| Total | 2.29 ± 1.52 | 0.20 ± 0.14 |

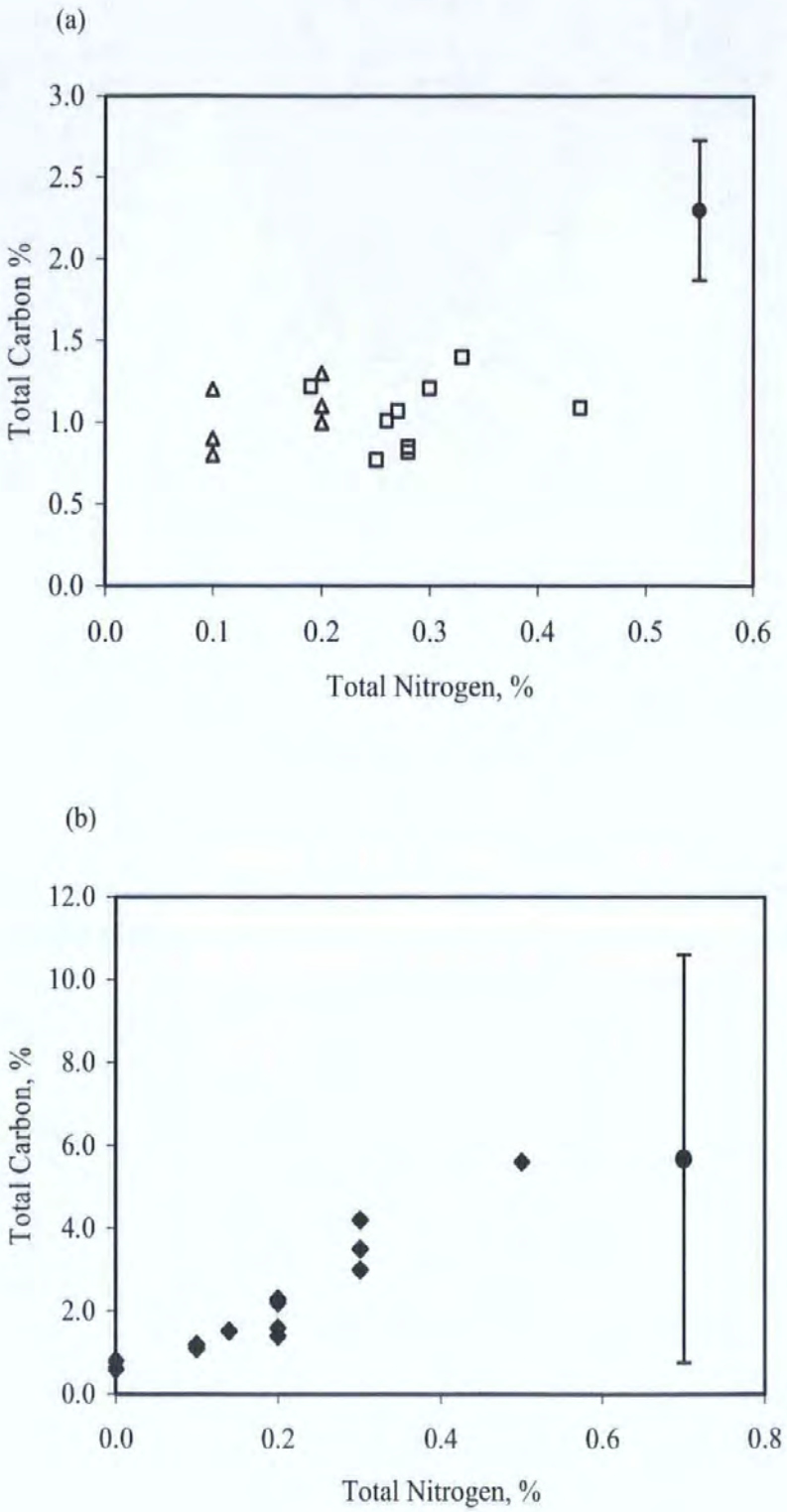


**Figure 3-13** **Total particulate carbon and nitrogen in the Pak Pa-Nang Estuary. (a) sediments (b) suspended particulate matter. □ -August 2001; Δ -December 2002.**

- **Nutrients and chlorophyll**

The range of concentrations of dissolved phosphate found for the Pak Pa-Nang are similar to those found in temperate estuaries in the UK[16, 17], with the exception of the Humber Estuary[18-20], which has higher values because it drains from a catchment that is affected by agricultural activities. The concentrations of dissolved phosphate generally declined with an increase in salinity, although the mean concentrations of phosphate in the freshwaters (Fig. 3.14a) were lower than those in the lower salinity region. The results show that in December 2002 and 2003 that dissolved phosphate appeared to be non-conservative in the low salinity region and the data for August 2001 show increased values in seawater. Removal of dissolved phosphate at low salinity has been observed previously in the Tamar Estuary, UK[16] and this has been attributed to uptake onto particles. The results for August 2001 show that at higher salinities the concentrations of dissolved phosphate increased, which may be due to a combination of the release of phosphate from particles, because of an increase in salinity or as a consequence of the mineralsation of carbon, and the inputs of domestic waste in the more populated areas around the coast of the Pak Pa-Nang.

Table 3-4 gives the mean and standard deviations of the dissolved phosphate concentrations at various times during the three surveys. Generally, the phosphate concentrations were in the range 0.62 to 0.96 $\mu mol\ l^{-1}$, with the exception of 2002 when values were about half those at other times. The lower values could be due to phytoplankton uptake, since the chlorophyll values were highest in 2002. Also, the relatively high dissolved phosphate concentrations in 2003 were associated with low chlorophyll concentrations. This could be due to the fact in 2003 the estuary was more turbid (see Table 3-1) and the phytoplankton growth could have been inhibited by low light levels. However, it is also noted that waste discharges from shrimp farms are likely to contain high levels of phosphate (Table 3-4), which may contribute to elevated levels in the estuary.

The concentrations of nitrate+nitrite are shown in Fig. 3-14b and it was found that nitrate was more than 85% of the combined total. The nitrate+nitrite concentrations in riverine water were lower than those in the low salinity waters, particularly along the western shoreline. Overall the concentrations varied over a narrow range from about 4

**Table 3-3 Mean ± standard deviation of salinity, nutrients and chlorophyll for the three surveys of the Pak Pa-Nang River Basin.**

| Location/Time | Salinity | Dissolved Concentration, μmol $l^{-1}$ | | | Chlorophyll |
|---|---|---|---|---|---|
| | | Phosphate | Nitrite+Nitrate | Silicate | μg $l^{-1}$ |
| Estuary 2001 | 26.9±2.7 | 0.88±0.96 | 3.68±5.47 | 21±13 | --- |
| Rivers 2002 a/r | --- | 0.62±0.41 | 7.82±4.73 | 170±27 | --- |
| Estuary 2002 b/r | 9.9±6.7 | 0.32±0.32 | 5.92 ± 5.07 | 69±23 | 27±19 |
| Estuary 2002 a/r | 9.4±7.1 | 0.47±0.56 | 6.76 ± 3.72 | 100± 48 | 21±19 |
| Rivers 2003 b/r | --- | 0.96±0.86 | 4.09±3.92 | 368±210 | 3.9±2.1 |
| Estuary 2003 b/r | 13.2±7.6 | 0.94±0.82 | 3.08±2.77 | 52±33 | 6.0±3.8 |
| Shrimp Farm | --- | 1.1 | 51 | 23 | --- |

b/r –before rain; a/r -after rain.

to 8 μmol $l^{-1}$ (Table 3-4). The inputs from shrimp farms could be important in maintaining relatively high nitrite+nitrate concentrations in the estuary. Compared to the concentrations of nitrite+nitrate and nitrate in temperate estuaries[16-20], the concentrations in the Pak Pa-Nang are low. In many cases, concentrations of dissolved nitrate and nitrite+nitrate show a linear relationship with salinity[16-18], with no addition or removal. Although there is general decrease in concentrations in Figure 3-14b there is no clear linear trend with salinity, which may be due to the bankside inputs, such as those from shrimp farms. Tappin[21] has pointed out that there has been a global growth in the discharges of N compounds from mariculture and this may have a long-term detrimental effect on water quality. Whether a long-term enrichment of the estuary is taking place[22] cannot be predicted because there is no historical database for nutrient upon which an assessment could be made. This should be a matter that the Irrigation Department should consider as part of its future management plans.

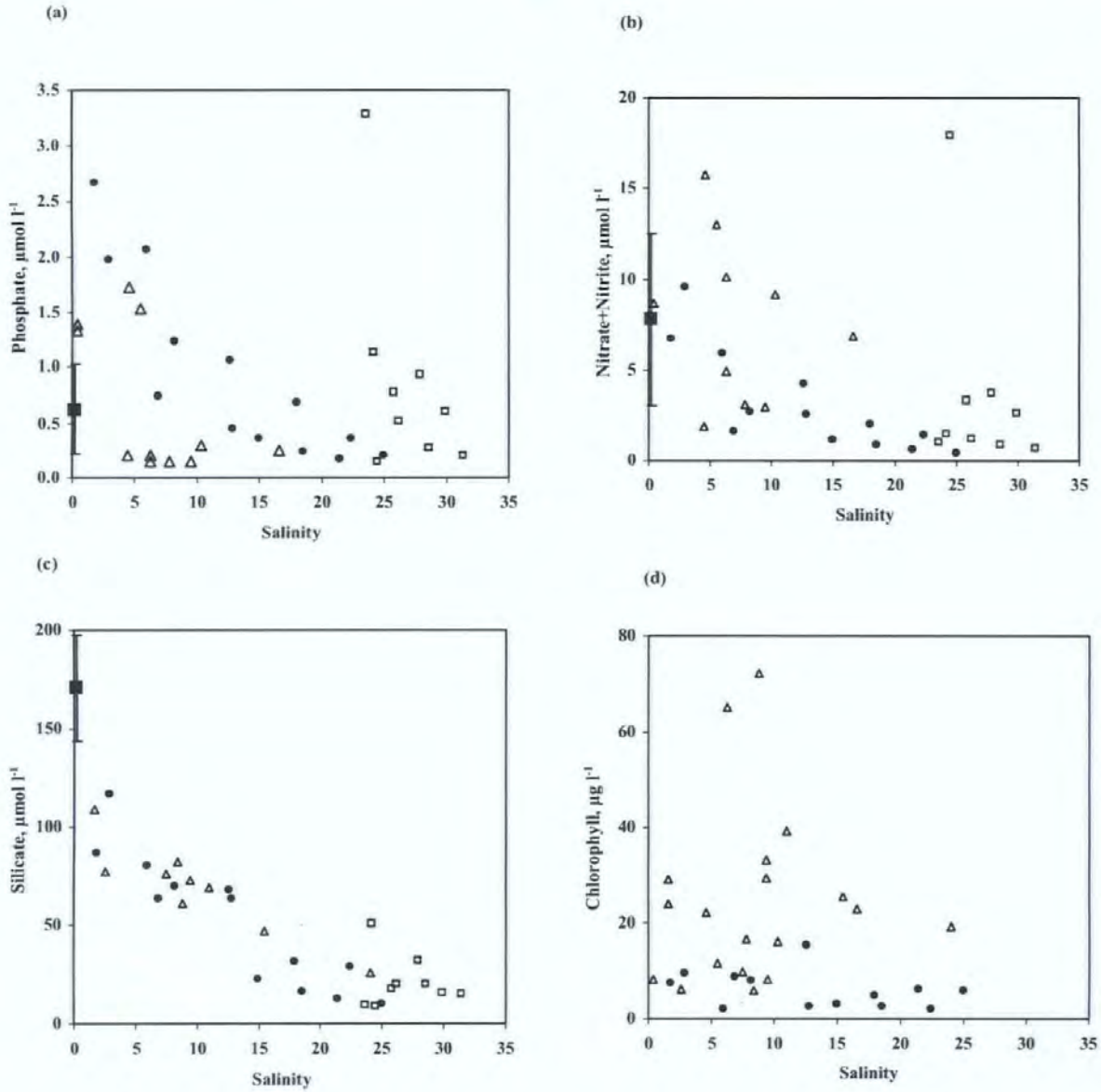


**Figure 3-14** **Nutrients and chlorophyll as a function of salinity in the estuary. □ -August 2001; Δ -December 2002; • -December 2003 (a) phosphate; (b) nitrate+nitrite; (c) Silicate; (d) chlorophyll. ■ –mean and standard deviation of riverine concentrations.**

The mean concentration of riverine silicate is higher than concentrations in the estuary, possibly due to weathering in the catchment. The silicate concentrations are similar to those found in temperate estuaries[16-20], where there appear to be no man-made sources of silicate. In the Pak Pa-Nang dissolved silicate concentrations show a linear relationship to salinity, as they do in some temperate estuaries[17,18]. Table 3-3 shows that dissolved silicate concentrations in the estuary respond to changes in the supply from the rivers. Taking 2003 as an example, when the rivers and estuary were sampled before the rain the mean dissolved silicate concentration in the rivers was about 7 times higher than in the estuary. Thus, during low river flow the relatively small flux of silicate to the estuary was diluted in seawater by a factor of seven. In 2002, before the rain the estuary concentration of silicate was comparable with that in 2002, i.e. it was 69 ± 23 μmol $l^{-1}$. After rain the concentration in the estuary increased to 100 ± 48 μmol $l^{-1}$, which was supported by a river concentration of 170 ± 27 μmol $l^{-1}$ and a greater flux of freshwater.

In 2003, prior to any flooding the chlorophyll concentrations were relatively low with a mean of 3.9 ± 2.1 μg $l^{-1}$, whereas following the floods the mean concentrations increased to 6.0 ± 3.8 μg $l^{-1}$. The highest chlorophyll concentrations were observed following in 2002 (Figure 3-14d). The difference in chlorophyll concentrations could be related to the fact that the water was more turbid in 2003, due to general monsoonal weather conditions and that low light levels inhibited phytoplankton growth.

### 3.5 Summary of the study of hydrography in the Pak Pa-Nang River Basin

Overall of the hydrography of the Pak Pa-Nang River Basin indicates that the riverine waters, feeding into the estuary, had a lower quality compared to waters in the estuary. Dissolved oxygen saturations had a mean value of 54% in riverine water which was lower than the estuarine water, with values between 80 and 100%. This is probably because of the oxygen demand due to the high carbon content of the riverine SPM, with values of total carbon up to 21%. The quality of the riverine waters is also affected by the nutrient content, particularly of nitritre and nitrate, where concentrations are increased due to uncontrolled inputs from nutrient-rich waters from shrimp farms, paddy fields and domestic wastes. The closing of the Pak Pa-Nang River Barrier also had an affect on water quality due to a lack of water exchange. This contributed to the decline of water quality, also observed by those living close to the water, and an absence of small fish. The concentration of nutrients, dissolved oxygen and pH were not

that much different to temperate estuaries but the need for water treatment plants in urban areas is now growing in importance. Thus, as part of the water management plan, water treatment should be considered as part of its future.

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# Chapter 4

# Arsenic in the Pak Pa-Nang River Basin

## 4.1 Arsenic distribution from the Ronpiboon mining area to the Pak Pa-Nang Estuary

This chapter contains the results of total arsenic and arsenic speciation analysis in the Pak Pa-Nang River Basin samples of sediment, water, SPM and biota. It is stressed that the majority of the concentrations have been expressed as the means and standard deviations of the analysis of three separate replicates sample. This approach was taken to ensure the quality of the data reported in this study.

The transportation of arsenic species in terms of the suspended particulate matter and as dissolved arsenic, especially in the more toxic inorganic forms ($As^{III}$ and $As^{V}$) has been studied. The effects from flood-waters downstream from the mountain-mining area through canals and rivers to the surrounding region has been of particular concern. This contaminant transport to the wider area, especially in the monsoon season, would have the potential to distribute arsenic to rice fields, shrimp farms and the fertile estuary and pose harm to people who consume those food resources.

### 4.1.1 Arsenic in sediment samples from the Pak Pa-Nang River Basin

- **'Total' arsenic in sediment samples**

Concentrations of arsenic in surface sediment samples from the Pak Pa-Nang River Basin were obtained from two samplings, December 2002 and December 2003. The

distribution maps are shown in Figure 4-1 and the full tabulated data is presented in Appendix A.

The sediment samples collected in the study area were found to have a variety of concentrations, ranging from 3 μg $g^{-1}$ in an uncontaminated to nearly 300 μg $g^{-1}$ in the most contaminated area. The highest concentration, 285 μg $g^{-1}$ was found in the 2002 sampling survey, station A4 (Namkhun Canal), which directly drains from the vicinity of the mine. In the December 2003 sampling survey, a similar distribution was found, where the maximum value of arsenic 177±7 μg $g^{-1}$ was found again at the station A4, and a concentration of 181±4 was found in a small stream (station M1) in the mining area, (see Figure 4-1). The source of arsenic running to the basin was found to originate from the mining area, as shown by the clearly visible higher concentrations of arsenic in sediments found at the west end of the basin.

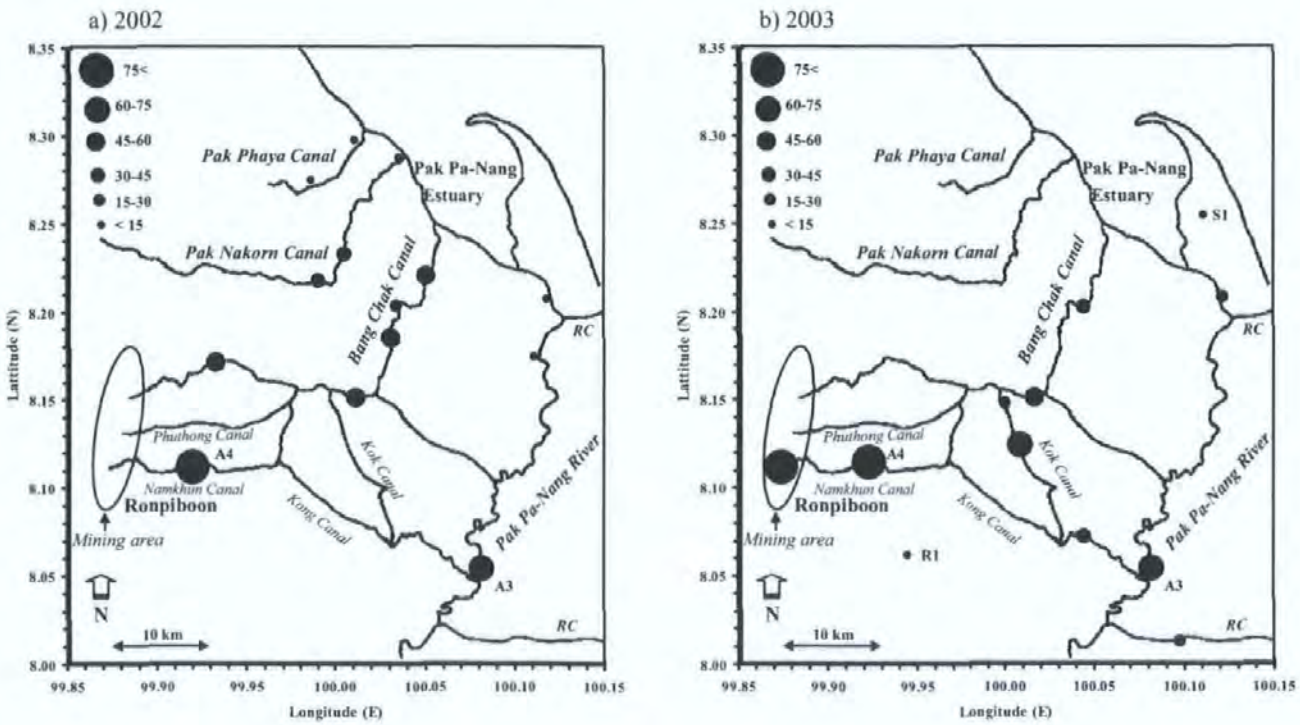



**Figure 4-1 Distributions of 'total' arsenic in surface sediments (stations are identified in Figure 3-2) of the Pak Pa-Nang River Basin; a) 2002 sampling; b) 2003 sampling, the units are in μg $g^{-1}$; S1 –sample from shrimp farm; R1 –sample from a paddy field; RC –flood relief channel to the Gulf of Thailand; (n=3).**

Apart from the Namkhun Canal, most of the elevated arsenic concentrations in sediments were found in the Bang Chak Canal and Kong Canal, ranging from 24 to 51 μg $g^{-1}$ and 24.5 to 60 μg $g^{-1}$, respectively. In the Pak Pa-Nang River sediments, arsenic concentrations were at 'normal' levels found in relatively uncontaminated sediments at

other sites, elsewhere. Concentrations of arsenic in 'uncontaminated' sediments are shown in Table 1-4, Chapter1. These ranged from 13 to 19 μg $g^{-1}$, except at the station A3 where a high concentration of 75 μg $g^{-1}$ was observed. This could be evidence that this station was associated with arsenic input from the Kong Canal and Kok Canal.

The Pak Phaya Canal is the most northerly of the rivers and it does not drain from a contaminated area. It is considered to relatively free of arsenic contamination, since the arsenic concentrations in sedments were 3 to 4 μg $g^{-1}$. The total concentrations of arsenic in soils from the region[1] have been put at 4 to 5 μg $g^{-1}$. This gives the general background concentrations of arsenic in the sediments for the River Basin as a whole. The values are significantly less than those from the contaminated region.

In addition to surface sediments, core sediment samples were collected from seven stations in the river basin during the 2002 and 2003 samplings (section 3.2.4; sediment samples). The core sampling covered the mid-part of the river basin (Figure 4-2) giving details of arsenic deposition over time. The core sampling took place in the wet season during relatively high river flows. The depth of the sediment samples collected being from 6 to 18 cm.

Figure 4-3 shows vertical distributions of 'total' arsenic in the seven core sediment samples taken from the Pak Pa-Nang River Basin. It was confirmed that the concentrations of arsenic in the sediments of the Bang Chak Canal (stations B3 and B4) and the Pak Pa-Nang River (A3) have been elevated over time and mean concentrations of 47±1.8, 49±3.4 μg $g^{-1}$ and 63±9 μg $g^{-1}$ were found at those stations, respectively. Lower values were found in the sediments further away from the mined area, for example station C1 (8.6±1.1μg $g^{-1}$) and A1 (13±1.3 μg $g^{-1}$) although C3 (43±5 μg $g^{-1}$) was higher. In 1996, Arrykul et al.[1] reported arsenic values from the region which were in reasonable agreement with those reported in this study. The authors also observed a general transport of arsenic from the mining area, but to a lesser extent than this study.

The vertical distributions of 'total' arsenic content were almost constant in the sediment cores at the stations C1, A1, B3 and B4 compared with stations C3 and A3, which showed evidence of variable deposition of arsenic and particularly the seasonal migration of arsenic from the source.

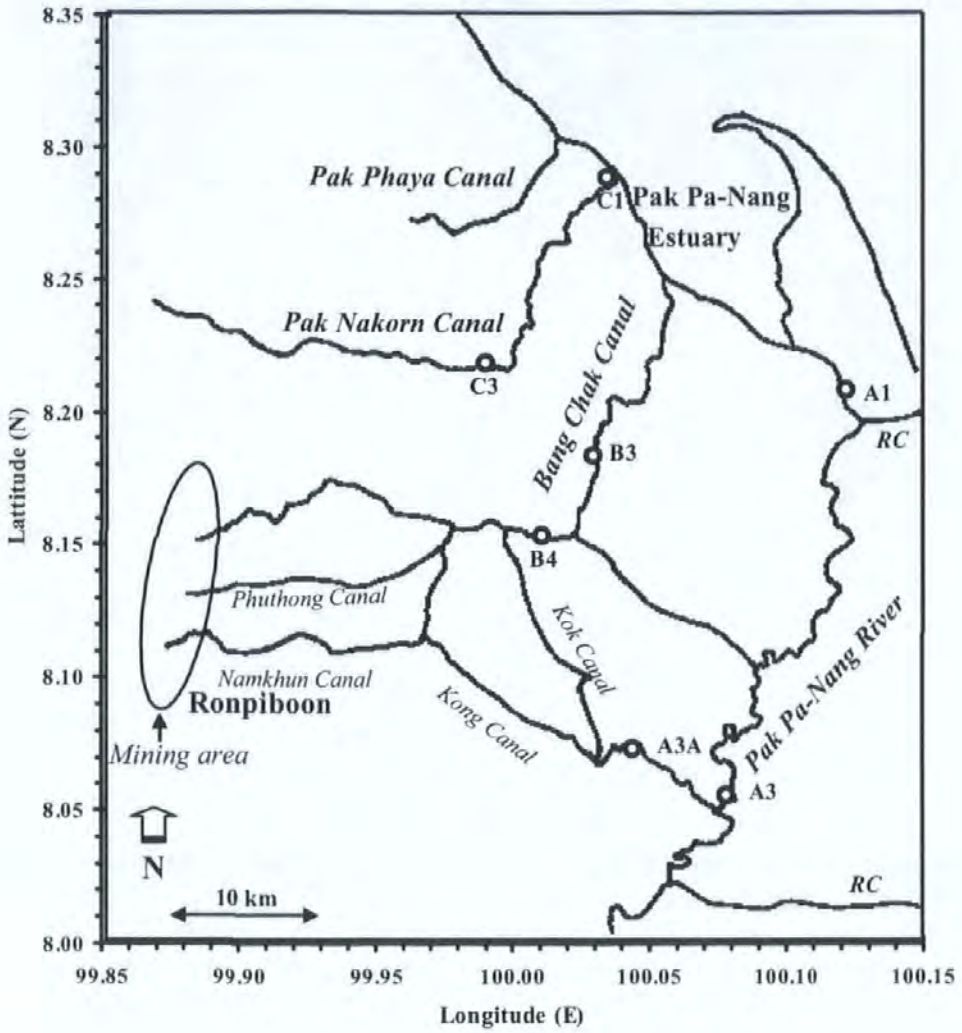



**Figure 4-2 Sediment core sampling stations in the Pak Pa-Nang River Basin; RC –flood relief channel to the Gulf of Thailand.**

The values observed at stations C1 and A1 indicate that reduced arsenic deposition occurs at these sites compared with the other sites further up stream. The relative water flows and particle size characteristic of SPM passing these sites may be instrumental to the lower, near-constant values of arsenic measured. The differences seen in the accumulation of arsenic with time between stations B3/B4 and C3/A3 reflect the different hydrographic cycles experienced at these sites. Apart from stations B3, B4, C3 and A3, where high concentrations of arsenic were found in core sediments, A3A is a site of concern. Increasing arsenic deposition during recent years was observed. In this core sediment, the total arsenic concentration was found to be approximately 13 $\mu g\ g^{-1}$ between 8 and 14 cm depth. Above 8 cm depth, the concentration was seen to continuously increase toward the surface, where the highest arsenic value of 34±4 $\mu g\ g^{-1}$ was obtained.

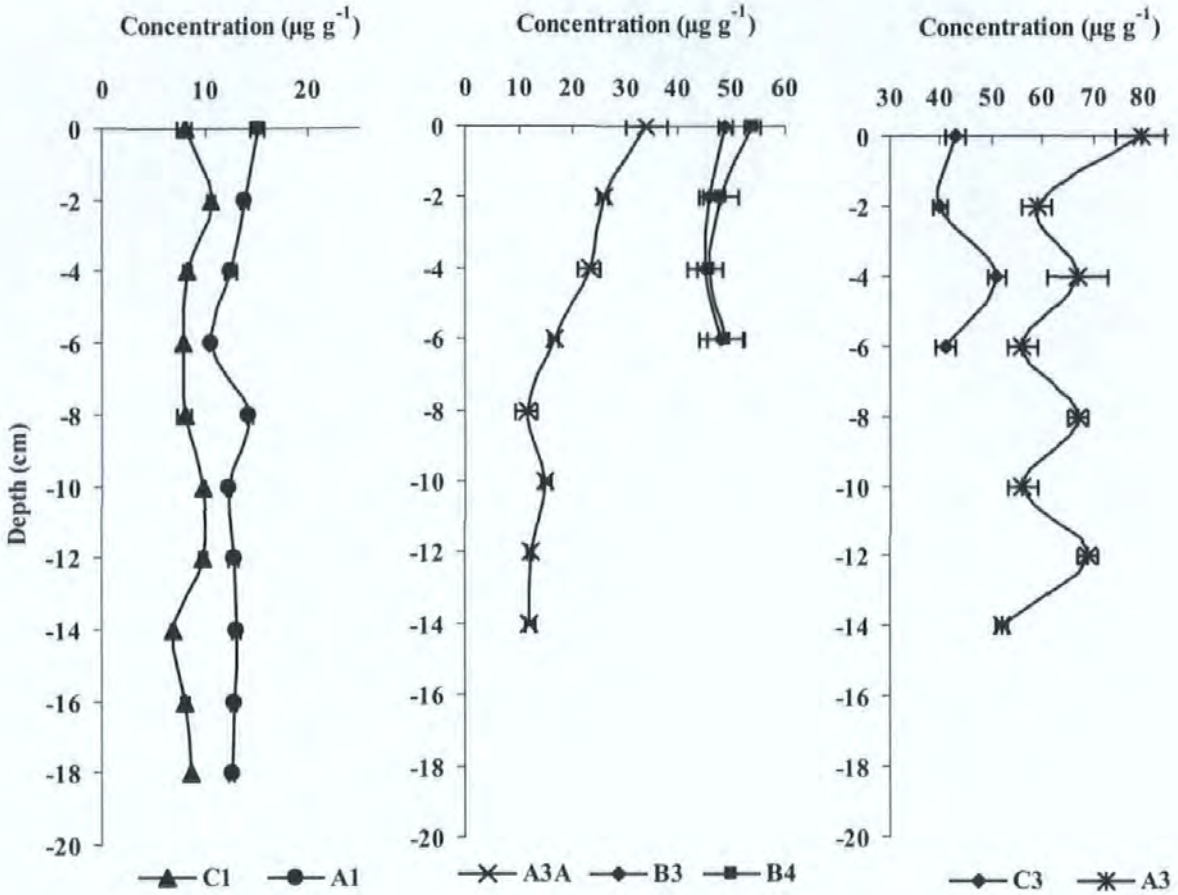



**Figure 4-3 Vertical distributions of total arsenic in core sediment samples taken from the Pak Pa-Nang River Basin (n=3).**

It is noted that water flows have been re-directed along the Kong Canal in recent times. This effect of arsenic accumulation at site A3A may be attributed to the diversion of water from the Namkhun and Kong Canals which are fed from the mining area.

Site A3 also shows a pattern of regular variations in the concentration of arsenic. The highest value is at the surface followed by a minimum concentration at 2 cm depth. The concentration then increases towards 4 cm depth, followed by another decrease and so on. This could be due to a seasonal pattern of deposition in the dry season followed by erosion in the wet season. Thus, there is a record of the annual cycle in the sediment column at station A3. Assuming a sediment depth of 4 cm in the core is equivalent to one year, according to this hypothesis so 8 cm depth of the sediment is equal to two years of deposition, which relates to the year of the re-routing of the water along the newly dug Kong Canal. In other words, the data from A3 gives an indication of the annual cycle which can be used to explain the gradual increase of arsenic concentration above 8cm depth of the sediment at station A3A.

- **Arsenic species in sediment samples**

Surface riverine sediments samples collected in December 2002 and December 2003 were also analysed for the arsenic species, $As^{III}$, $As^{V}$, DMA and MMA. The results are shown in Table 4-1.

**Table 4-1 'Total' arsenic [mean ± standard deviation, n=3; (range)] and percentage of arsenic species in riverine sediments.**

| Sampling | Arsenic concentrations and species content | | | |
|---|---|---|---|---|
| | 'Total' As ($\mu g\ g^{-1}$) | Inorganic $As^{III}$ (%) | Inorganic $As^{V}$ (%) | MMA (%) |
| December, 2002 (n=14) | 50±71 (3-285) | 3-14 | 84-97 | nd-3 |
| December, 2003 (n=12) | 58±60 (7-181) | 2-7 | 93-98 | nd-2 |

nd= not detectable, the detection limit for MMA = 0.12 $\mu g$ As $g^{-1}$.

Inorganic arsenic, $As^{V}$ was found to be the major species, ranging from 84-98% of content, with the minor inorganic species $As^{III}$, having a range of 2-14%. The organo arsenic species, MMA was found in small quantities, ranging from below the detection limit up to 3% of the total arsenic content. DMA was not detected in any sample. The arsenic species in sediments found in the study area are comparable to the normal composition of arsenic species found in sediments from other areas, as concluded by Francesconi and Edmonds (1997)[2] (see Table 1-9, Chapter 1).

To obtain a profile of the vertical distribution of arsenic species in a sediment core, a whole sediment column from the Pak Pa-Nang River, station A3, was analysed for arsenic species. The 'total' arsenic concentration and percentage of arsenic species are shown in Figure 4-4. Inorganic, $As^{V}$ was the major arsenic species found, ranging from 86 to 93% of content with minor species of $As^{III}$ ranging from 5 to 12%. MMA was found in small quantities within the range 1-3%. The decrease in inorganic $As^{V}$ is clearly shown with increasing depth. This profile is indicative of a process of reduction, which increases with depth and may show inorganic $As^{V}$ transforming to inorganic $As^{III}$. The decline in expected oxygen content with depth together with the presence of

organic matter and the change in the MMA content is suggestive of a mechanism involving a direct route to inorganic $As^{III}$ via methylation and demethylation processes.

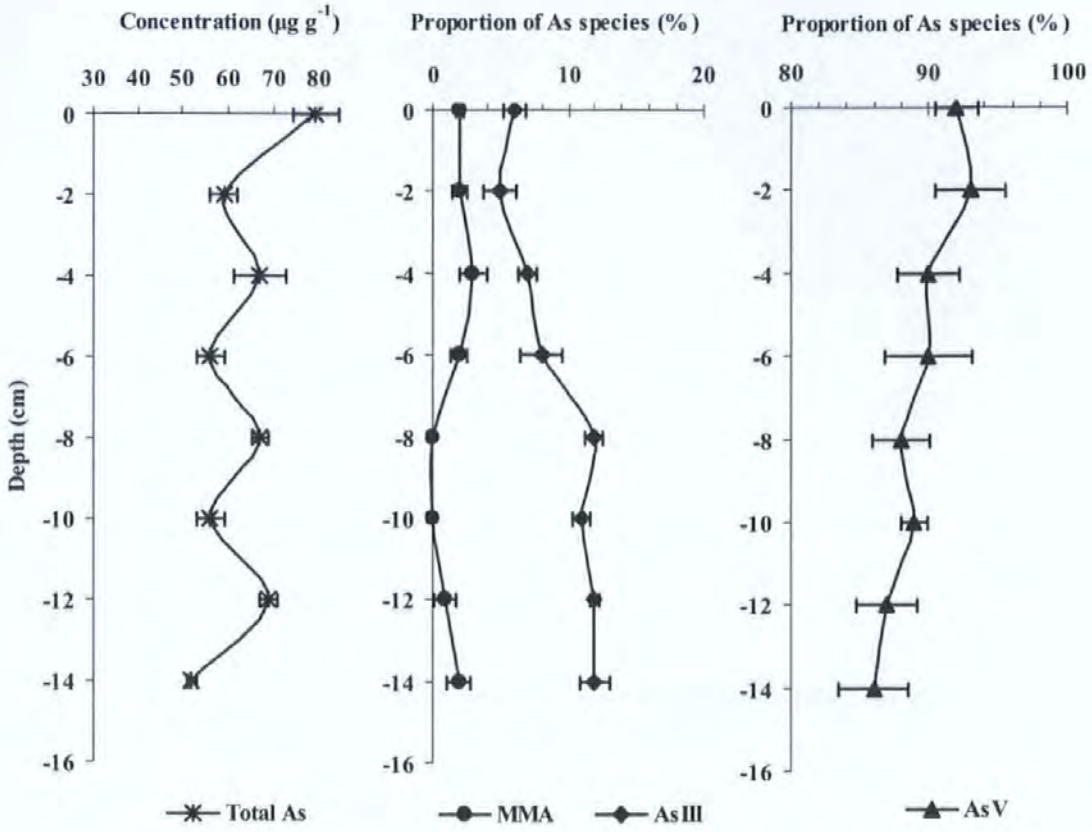



**Figure 4-4 Vertical distributions of 'total' arsenic and arsenic species in the sediment column from station A3 (n=3).**

- **Associated trace metals in sediment core samples**

The core sediment samples taken from station A3 were also analysed for the trace metals Cu, Ni and Pb. This was undertaken in order to investigate the levels of those elements that can associate with arsenic deposits and therefore offer further evidence of transport from the mining area. The mean concentrations for Cu, Ni and Pb were found to be 24±7.8, 12±6.7 µg $g^{-1}$ and 10±4.6 µg $g^{-1}$, respectively. The depth profiles are shown in Figure 4-5. At depths from 8 to 14 cm, the Cu and Ni profiles show some similarity to arsenic. While it may be suggested that Cu and Ni might be associated and hence transported along with arsenic (because of the past mining activity) it is noted that the profile for Pb only followed that of arsenic from the surface down to 6 cm depth.

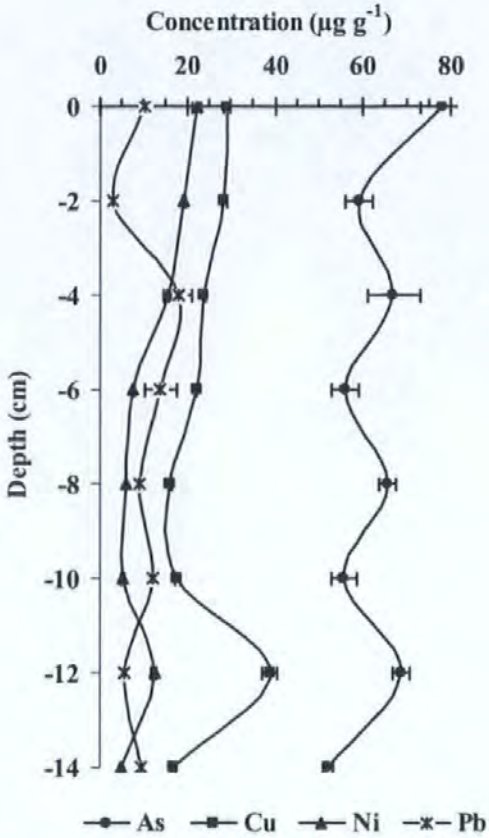



**Figure 4-5 Vertical distributions of total trace metals in core sediment samples taken from the station A3 (n=3).**

The Pb concentrations measured in this study were low and similar to those found in surface sediments by Arrykul et al.[1]. The highest values found by Arrykul et al.[1] were in the contaminated area and at station A3, they reported < 20 μg $g^{-1}$ of Pb. In the lower Pak Pa-Nang, all the Pb concentrations were also reported < 20 μg $g^{-1}$.

### 4.1.2 Arsenic in water and SPM samples from the Pak Pa-Nang River Basin

- **Dissolved arsenic in riverine water samples**

The dissolved arsenic values in riverine water samples collected in December 2002 and December 2003 were found to be in a concentration range of 1.7-120 μg $l^{-1}$and 1.2-94 μg $l^{-1}$, respectively (Figure 4-6 and full tabulated data is presented in Appendix A). These values were often a reflection of the arsenic distributions in sediment; the highest dissolved arsenic concentrations in both surveys were found at the station A4 (Namkhun Canal), which drains from the mining area. This was a result of flooding that

was taking place during the survey and the possible release of high arsenic concentrations from the mining area. In 2003, a water sample was collected from a mountain stream in the mining area (station M1), where a concentration of 148 μg $l^{-1}$ was found.

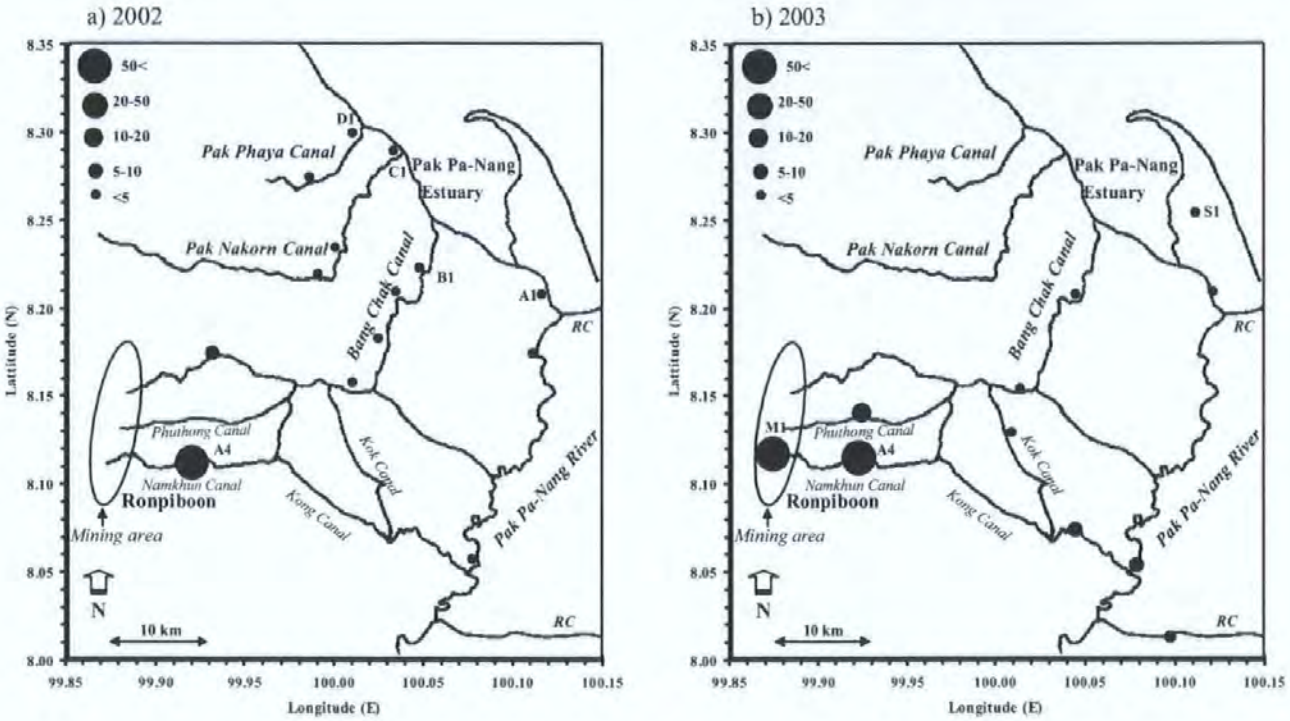



**Figure 4-6 Distributions of dissolved arsenic in the Pak Pa-Nang River Basin, a) 2002 sampling, b) 2003 sampling; the units are in μg $l^{-1}$; S1 –sample from shrimp farm; M1 – sample from a mountain stream in the mining area; RC –flood relief channel to the Gulf of Thailand.**

Slightly elevated concentrations of dissolved arsenic were found in the Bang Chak and Kong Canals compared with other rivers found in the 2002 and 2003 surveys. The concentration increase was most probably due to the effect of the higher arsenic concentrations from the Namkhun and Phuthong Canals.

In general, the concentrations of dissolved arsenic in the rivers and canals were found to be at moderate levels as observed in relatively uncontaminated rivers (see concentrations of arsenic in other rivers in Table 1-6, Chapter 1) except in the canals near the mining area. At the river mouths to the estuary, the dissolved arsenic concentrations of 2.4 μg $l^{-1}$, 4.1 μg $l^{-1}$, 3.3 μg $l^{-1}$ and 3.9 μg $l^{-1}$, were found in the stations A1, B1, C1 and D1 from the 2002 sampling, respectively. These concentrations will be discussed later when comparing the dissolved arsenic concentrations in the estuary.

- **Dissolved arsenic species in riverine water samples**

Water samples collected in December 2002 and December 2003 were analysed for total arsenic and inorganic arsenic. The results are shown in Table 4-2. The major form of dissolved arsenic was found in its inorganic form. The concentrations of dissolved inorganic arsenic found in the 2002 samples range from 70 to100% of the total arsenic. In the 2003 survey, inorganic arsenic was also found to be the dominant form, with the exceptions of those from stations A3 and A3B, where dissolved organic arsenic was found to be the dominant form, comprising 87% and 66% of content, respectively.

**Table 4-2 Dissolved arsenic species in water samples, mean ± standard deviation.**

| Study | Arsenic concentrations | |
|---|---|---|
| | Total arsenic ($\mu g\ l^{-1}$) | Inorganic As (%) |
| December, 2002 (n=14) | 12±32 | 85±13 |
| | (1.7-122) | (70-100) |
| December, 2003 (n=11) | 26±46 | 74±32 |
| | 1.2-148 | (13-100) |

- **'Total' arsenic concentrations in SPM**

Distributions of 'total' arsenic in SPM collected from the Pak Pa-Nang River Basin are shown in Figure 4-7 and the full tabulated data is presented in Appendix A. The concentrations in SPM collected in December 2002 were found to have a concentration range of 6.1-300 $\mu g\ g^{-1}$. In December 2003 a concentration range of 8.4-840 $\mu g\ g^{-1}$ was found. The maximum concentrations of arsenic in SPM, sampled in December 2002 and December 2003, gave values of 298 and 836 $\mu g\ g^{-1}$, respectively and these high arsenic concentrations were found again in the Namkhun Canal, which drains directly from the mining area.

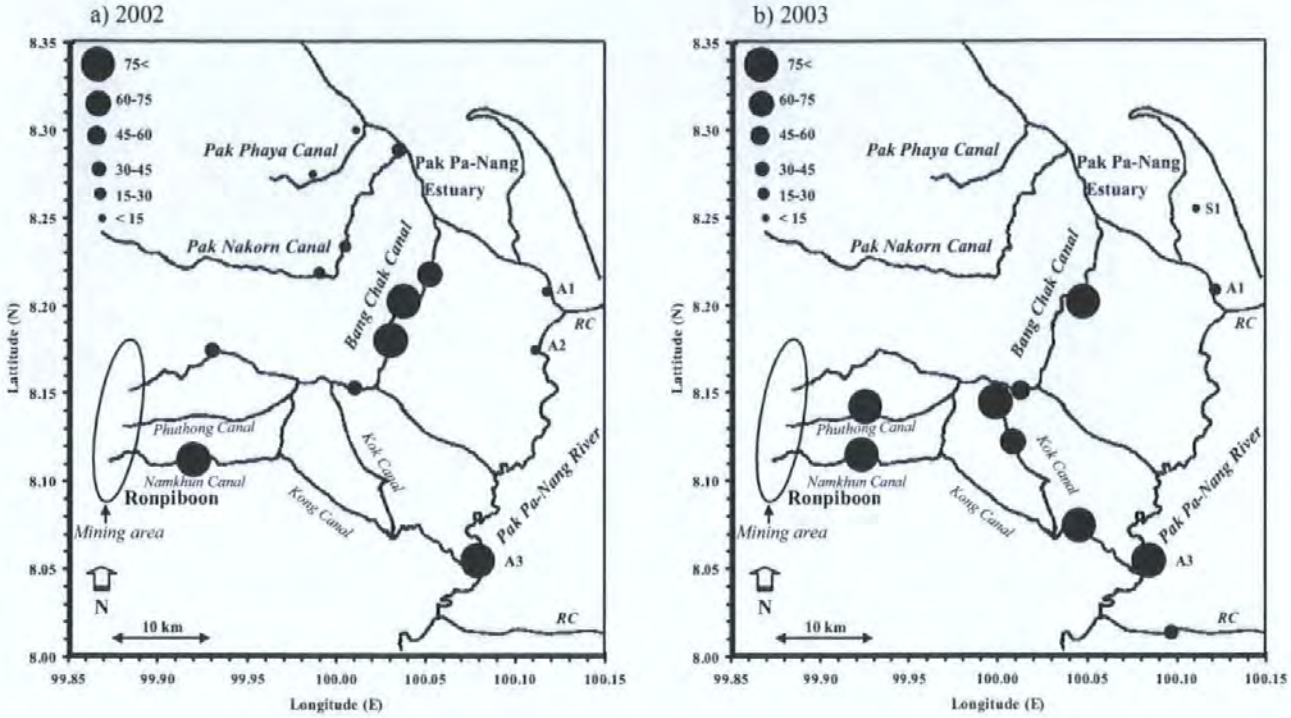



**Figure 4-7 Distributions of 'total' arsenic in SPM from the Pak Pa-Nang River Basin, a) 2002 sampling, b) 2003 sampling, the units are in μg $g^{-1}$. S1 –sample from shrimp farm; RC –flood relief channel to the Gulf of Thailand.**

In agreement with the arsenic concentrations found in the sediment samples, elevated concentrations were also found in the SPM from the Bang Chak Canal and also the Kong Canal. The high concentrations also found in the Pak Pa-Nang River station A3, were strongly evidenced as being derived from the Kong Canal. Arsenic in SPM from other stations in the Pak Pa-Nang River (A1 and A2) were not found to be as elevated. This is possibly due to high arsenic concentrations from the Kong Canal, which drains into the Pak Pa-Nag River, mixing with the greater volume of water flowing in the Pak Pa-Nang River, which had lower concentrations of arsenic in its SPM.

- **Arsenic species in SPM**

The speciation of arsenic in riverine SPM was only achievable with those samples that had a sufficiency high concentration of 'total' arsenic. The SPM samples collected from stations AB1, A4 and A3 in 2003 were analysed for the arsenic species, $As^{III}$, $As^{V}$, MMA and DMA. To study the seasonal effect upon the arsenic concentration and speciation in SPM, samples were collected at stations, AB1 and A4, before and after a heavy (monsoon) rainfall. These results are shown in Table 4-3. The major species of

arsenic found was $As^{V}$, in the range from 92 to 95%. The minor species were inorganic $As^{III}$, in the range from 3 to 4% and organic MMA in the range from 1 to 4%. DMA was not detected in any sample. The 'total' arsenic concentration was found to decrease in those samples that were collected after the heavy rainfall but the speciation compositions were found not to be effected by the rain.

**Table 4-3 Arsenic species in Riverine SPM samples taken before and after a heavy (monsoon) rainfall; total As=mean ± standard deviation (n=3).**

| Sample | Arsenic concentrations ($\mu g\ g^{-1}$ As) | | | |
|---|---|---|---|---|
| | Total As | Inorganic $As^{III}$ | Inorganic $As^{V}$ | MMA |
| Puthong Canal (before rain) (AB1) | 787±54 | 24(4%) | 556(94%) | 10(2%) |
| Puthong Canal (after rain) (AB1) | 180±9 | 6.2(4%) | 151(95%) | 2(1%) |
| Namkhun Canal (before rain) (A4) | 836±86 | 18(3%) | 644(93%) | 27(4%) |
| Namkhun Canal (after rain) (A4) | 263±27 | 8.5(4%) | 220(92%) | 10(4%) |
| Pak Pa-Nang River (before rain) (A3) | 149±3 | 6.6(4%) | 134(92%) | 5.6(4%) |

### 4.1.3 Arsenic distribution and speciation in rice plants (*Oryza sativa*)

It is important to recap, that as an agricultural society, Thailand has relied on rice farming since time immemorial. A development plan in Nakorn Si Thammarat proposed the construction of a dam in the Pak Pa-Nang River, which would greatly benefit the farmers on the upper river plain. A water management plan based on the dam was devised so that farmers in the area can use riverine water for irrigation in order that rice can be grown during all six months of the dry season. If the arsenic that is distributed from the contaminated area is transported in the fresh water, it could build up in the soil from the irrigation activities. There is therefore the potential of arsenic to be taken up by rice crops, and the exposure of people to arsenic in the area concerned, since the distribution of rice from mills is a local activity.

The concentration of 'total' arsenic and species found in the soil sample and in different parts of the rice plant such as the rice root, rice stem, rice leaf and rice grain are presented in the Table 4-4. The results show that root samples contained the highest accumulation of total arsenic compared with other parts of the plant. The arsenic content of the soil sample was 7.3±0.8 μg $g^{-1}$. Arsenate was determined as the major component, with lower levels of arsenite and MMA, and the percentage of arsenic in these forms were, 80%, 16% and 4%, respectively. Inorganic $As^{III}$ was promoted in the paddy soil under submerged soil conditions the percentage of $As^{III}$ found, 16% was greater than normal surface soils and sediments. There was evidence of arsenic methylation in paddy soil systems, where the transported inorganic species were converted to the organic form by micro organisms.

**Table 4-4 Arsenic in rice plant and paddy soil samples, total As=mean ± standard deviation (n=3).**

| Sample | Total arsenic [a] (μg $g^{-1}$) | MMA [b] (μg $g^{-1}$) | Inorganic arsenic [b] (μg $g^{-1}$) |
|---|---|---|---|
| Paddy soil | 7.3±0.8 | 0.6 | 7.5 |
| Rice root | 189±10 | 88 | 62 |
| Rice stem | 6.1±0.3 | 3.9 | 0.9 |
| Rice leaf | 4.6±0.14 | 3.3 | 0.6 |
| Rice grain 1 | 0.4±0.02 | 0.33 | nd |
| Rice grain 2 | 0.4±0.03 | 0.32 | nd |

[a] Values from acid digestion for total arsenic; [b] Values from enzyme extraction for arsenic species nd= not detectable, the detection limit = 0.15 μg $g^{-1}$.

Figure 4-8 shows arsenic concentrations (dry weight basis) and percentage of arsenic species in different parts of the rice plant. This figure shows that the roots of rice plant accumulated the greatest level of arsenic, followed by the stem, the leaf and the rice grain. The latter accumulated the least amount of arsenic. This trend is in agreement with that reported by Abedin et al.[3] based on a greenhouse study of arsenic accumulation in rice plants. The proportion of inorganic arsenic was reduced from root to stem to leaf and only MMA was found in the rice seed. This reduction may represent a methylation route for inorganic species from soil to root, root to stem and stem to leaves by the rice plant in this study. However, the possibility of the preferential

accumulation of MMA over that of inorganic arsenic must also be considered for each stage, i.e. soil to root, root to stem, stem to leaf and stem to grain.

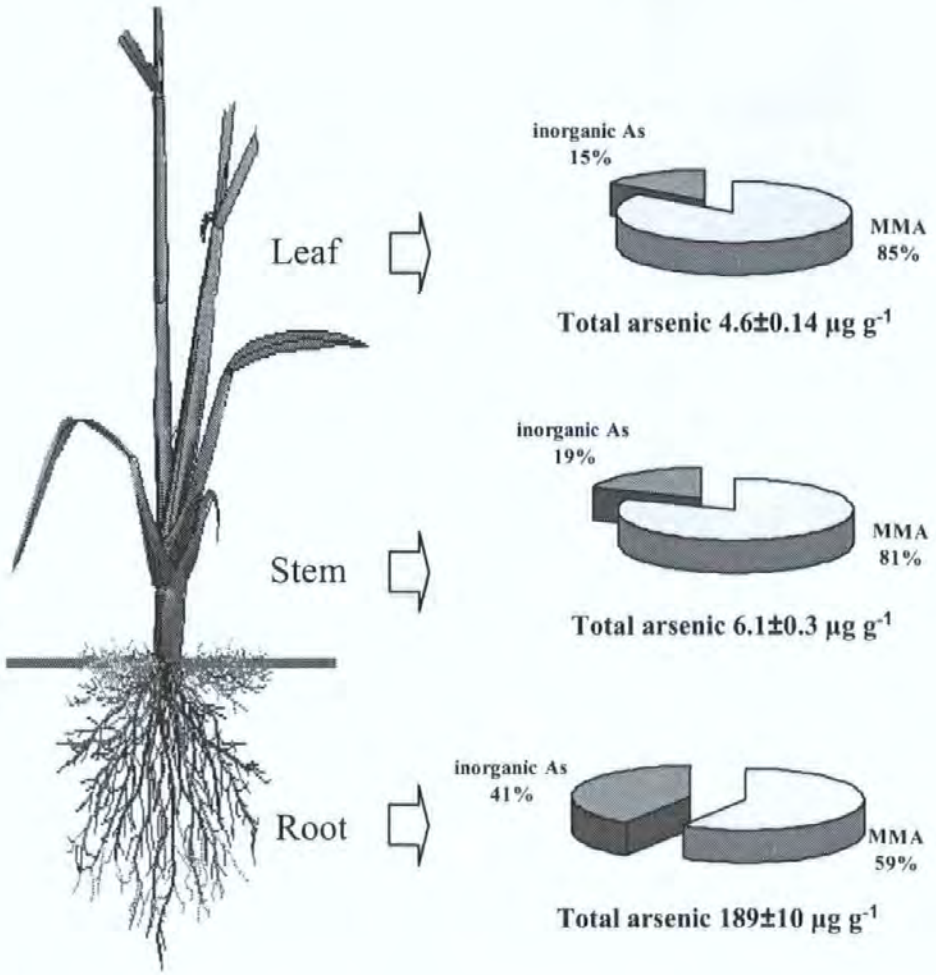



**Figure 4-8 Distribution and speciation of arsenic in rice plant (*Oryza sativa*), (dry weight basis).**

Growing paddy rice in arsenic contaminated soil or by irrigating rice with arsenic contaminated water can cause elevated arsenic concentrations in the aerial plant parts. The literature reports elevated arsenic concentrations in rice straw through absorption of arsenic in the soil[4,5]. Abedin et al., also observed a large accumulation of arsenic by rice plants, with comparable root and straw concentrations of 100 μg $g^{-1}$ arsenic where rice was irrigated with a solution containing 8 μg $l^{-1}$ arsenate[3]. Even though the arsenic concentration in rice straw was elevated, the accumulation in rice grains was limited and was less than the maximum permissible limit of 2 μg $g^{-1}$ ('total' arsenic wet weight basis), as set by the Ministry of Public Health, Thailand[6].

While the presence of arsenic in rice grain may be at relatively safer levels for the human consumer, the higher concentrations of arsenic in rice straw could pose a potential health hazard directly to the cattle population because rice straw is used as cattle feed in some local villages in the Ronpiboon area. This could also introduce arsenic into the human food chain though the consumption of cattle products e.g. milk, beef etc. A full mass balance for the rice plant analysis is presented in Table 4-7 and discussed in section 4.3.1 as part of the comparative efficiency for the different enzymatic extraction methods.

## 4.2 Arsenic concentrations in the Pak Pa-Nang Estuary

It is important to remember that the Pak Pa-Nang Estuary is a shallow, elongated basin, approximately 14 km long and 3 km wide at the mouth of the Pak Pa-Nang River up to about 10 km wide at the entrance to the Gulf of Thailand. It is situated in the northeast, approximately 70 km far from the Ronpiboon mines. The effect upon the environment of the estuary is of concern because of the spreading of arsenic from the mining area. Sediment, water and marine biota samples from the estuary were collected and analysed. These results are discussed in the following sections.

### 4.2.1 Arsenic in sediment samples

Surface sediments from nine sites in the estuary system were sampled in the dry season of 2001 and the wet season in 2002. The samples were analysed for 'total' arsenic and arsenic species. The concentrations found in the estuary were in the range found in relatively less contaminated aquatic sediments elsewhere (typical background concentrations of aquatic sediment 1-50 $\mu g\ g^{-1}$ have been reported[7,8]). Total arsenic concentrations in the estuary range from 6 to 14 $\mu g\ g^{-1}$ and 8 to 20 $\mu g\ g^{-1}$ in the dry season, 2001 and wet season, 2002, respectively (Figure 4-9 and 4-10 and full tabulated data is presented in Appendix A).

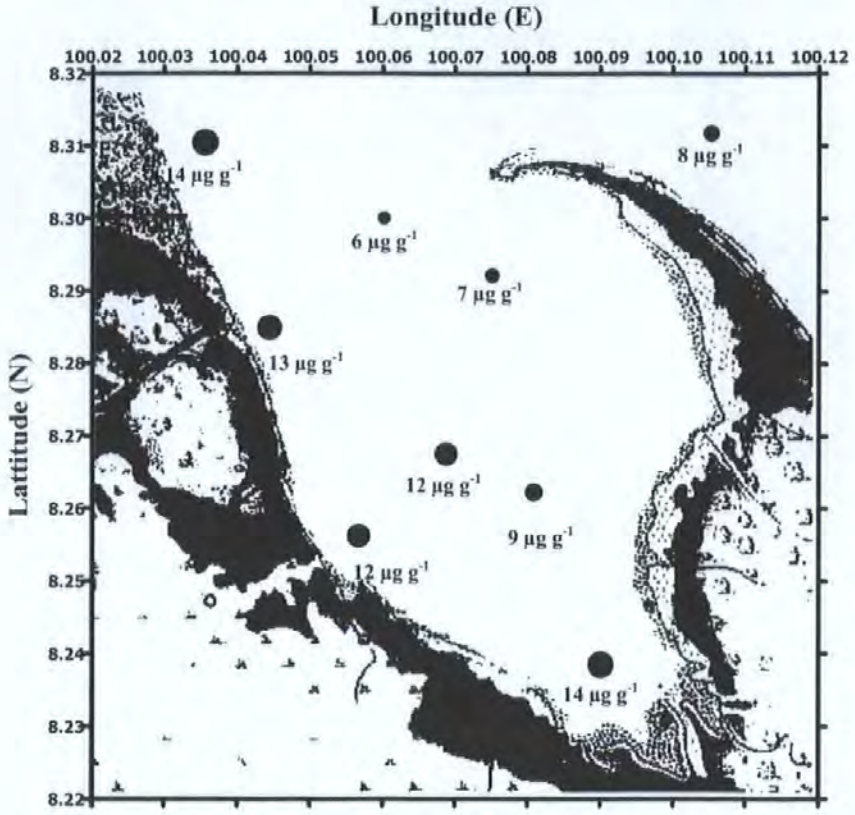



**Figure 4-9** Arsenic concentrations in surface sediment samples collected in dry season 2001 (n=3).

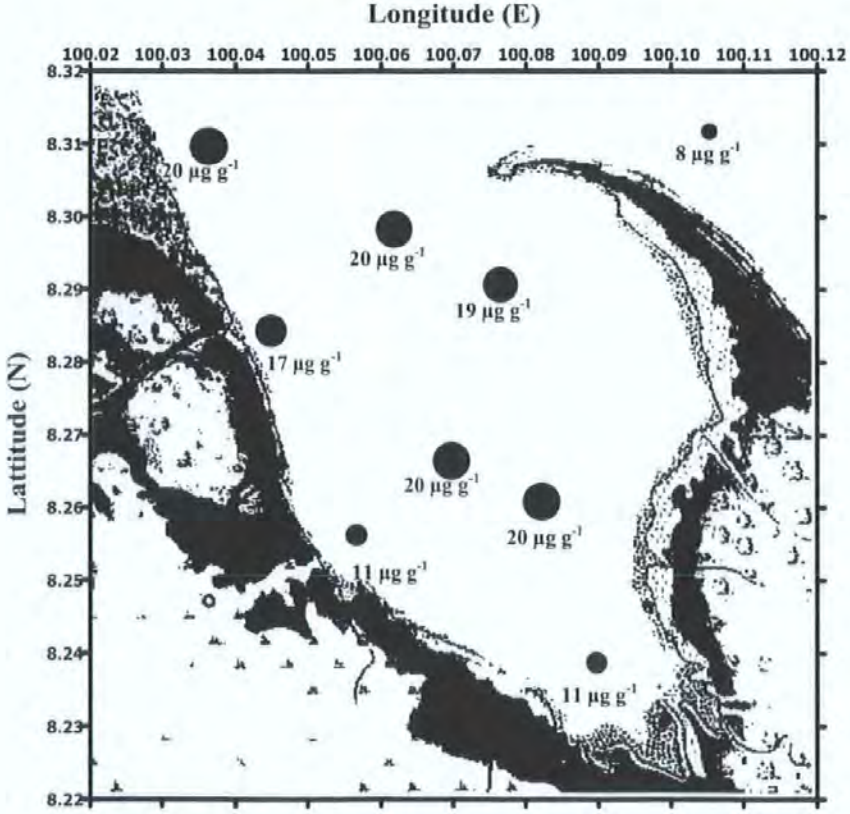



**Figure 4-10** Arsenic concentrations in surface sediment samples collected in wet season 2002 (n=3).

There was a decreasing concentration gradient outwards from the four river mouths and the lowest concentrations were found offshore in the dry season 2001 survey. Lower arsenic concentrations were obtained in the dry season compared with the wet season, with average concentrations of 10.4±3.6 μg $g^{-1}$ and 16.2±4.8 μg $g^{-1}$, respectively. In the wet season 2002 survey, the gradient of higher arsenic concentration was moved towards the centre of the estuary. This indicated that arsenic was removed from the water column and deposited further outward from the river mouths toward the estuary during the wet season (compare distributions in Figure 4-9 and 4-10); as would be expected during the higher discharges experienced during the wet season. On mixing with seawater, this deposition would be occurring further away from the river mouths during the higher discharges in the wet season (see wet season salinity profile of the estuary in Figure 3-8, Chapter 3). Deposition processes may be a consequence of reduced water dynamics and/or flocculation processes through the mixing of riverine water and seawater affecting the setting velocities of the particles[9].

Core sediment samples, ppn 1, ppn 2, ppn 3 and ppn 4 from the river mouths of the Pak Pa-Nang River, the Bang Chak, the Pak Nakorn and the Pak Phaya Canals, respectively (Figure 3-5, Chapter 3) were acquired and analysed from the 2002 survey. In addition a core sediment sample from the east of the estuary, station 35 (see Figure 3-5, Chapter 3), was also taken and analysed in 2003. Vertical distributions of arsenic in the sediment cores are shown in Figure 4-11. The 'total' arsenic concentrations found ranged from 6.3 to 30 μg $g^{-1}$. The trend in the vertical distribution of arsenic was found to be uniform at the surface to the depth of 6 cm for nearly all stations. Deeper than 6 cm depth, the distribution was found to vary slightly at different depths, except in station ppn 2 (Bang Chak Canal), where concentrations increased as depth increased. The concentration at 14 cm depth was found to be 3 times higher than the concentration at the surface. It is of note that core 35 also showed a higher concentration at 14 cm depth. This may represent a higher arsenic concentration being deposited during the period of high mining activity for tin in the past.

In addition to total arsenic analysis in the core sediment samples taken, those from stations ppn1, ppn3 and ppn4 were also analysed in order to investigate the levels of Cu, Ni and Pb. As stated previously, this was undertaken to measure those elements that can associate with arsenic deposits and therefore offer further evidence of transport from the mining area. The results are shown in Figure 4-12.

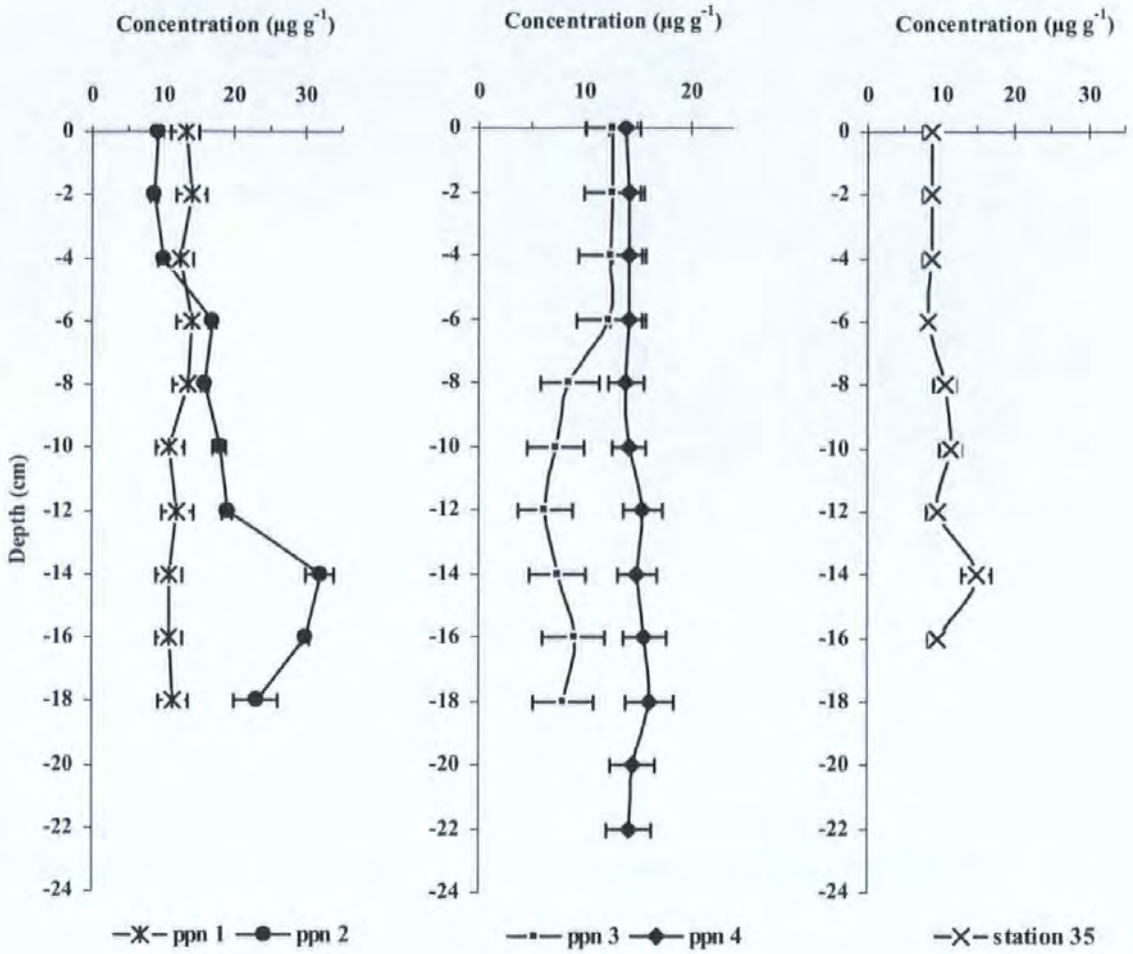



**Figure 4-11 Vertical distribution of total arsenic in core sediment samples taken from the Pak Pa-Nang Estuary; n=3 (stations are shown in Figure 3-3 and 3-4).**

The mean concentrations for Cu, Ni and Pb were found to be 9±0.5, 20±2 µg $g^{-1}$ and 25±6.5 µg $g^{-1}$, respectively. The Ni and Pb concentrations are higher than those concentrations found in the Pak Pa-Nang River at station A3 (Figure 4-5), being 12±6.7 µg $g^{-1}$ and 10±4.6 µg $g^{-1}$, respectively. This suggests that other sources of these metals are introduced to the estuary. The depth profiles for Cu show a similar vertical distribution to As with some slight variations. Ni depth profiles were found to show a seasonal variation, which also indicates the input of this metal is particularly from the Bang Chak and the Pak Nakorn Canals, ppn3 and ppn4. Pb concentrations were found to be higher in the estuarine sediments than those from the Pak Pa-Nang River but no sign of a seasonal variation in the river was shown. This may suggest an additional input from the use of leaded petrol which may have entered the estuary from the populated areas or from atmospheric deposition over many years.

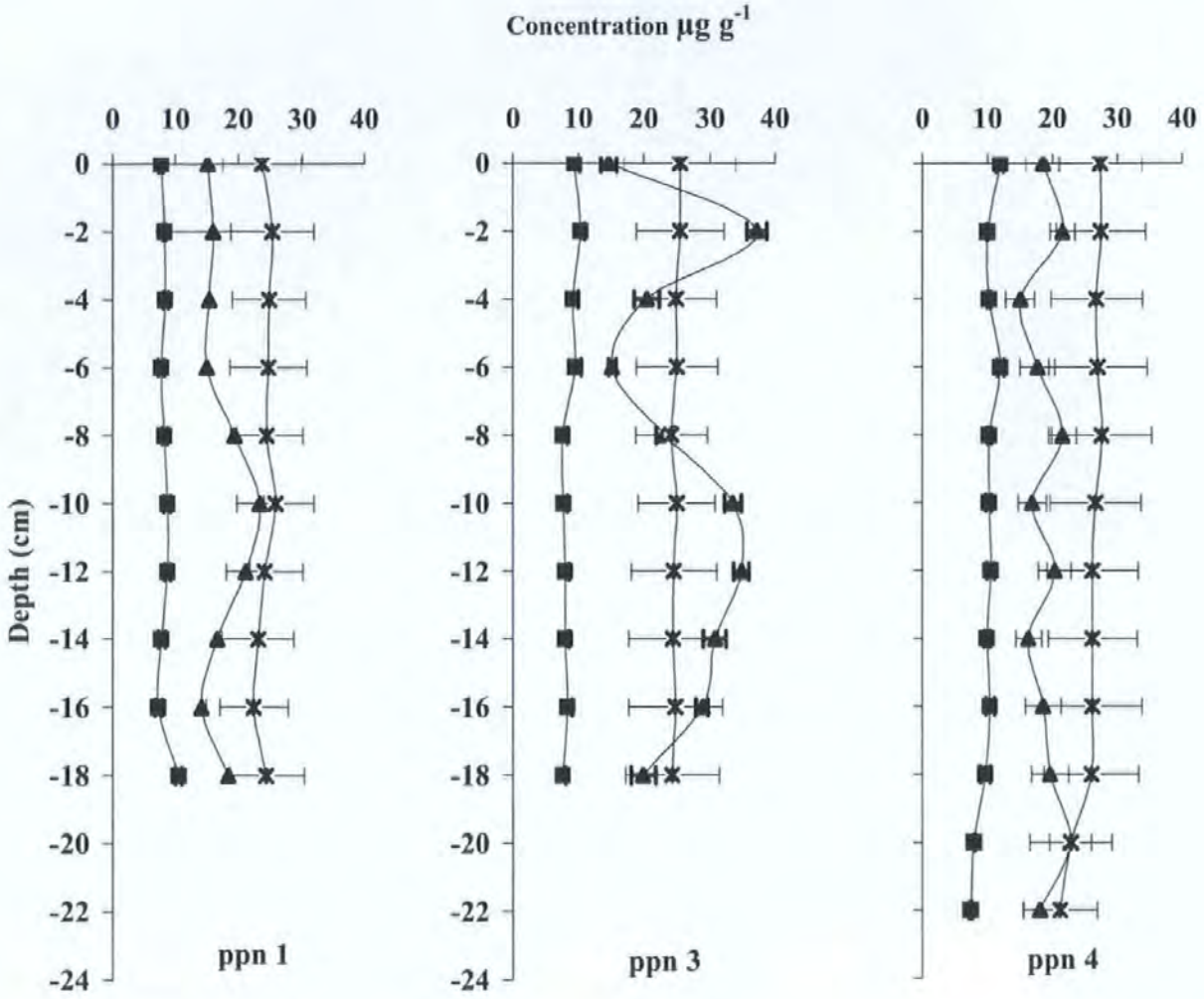



**Figure 4-12 Vertical distributions of total trace metals in core sediment samples taken from the station ppn1, ppn3 and ppn4; ■ –Copper; ▲ –Nickel; × –Lead; (n=3).**

- **Arsenic species in sediments**

The surface sediments of the Pak Pa-Nang Estuary were also analysed for arsenic species, and these results are shown in Table 4-5. Inorganic $As^{V}$ was the major species found and ranged from 80 to 90% and 92 to 94% in the dry season and wet season, respectively. Inorganic, $As^{III}$ was the minor species ranging from 10 to 14% and 6 to 8% of total arsenic in dry season and wet season, respectively. The organic arsenic species, MMA and DMA, were not found in any of the samples. The ratios of $As^{III}/As^{V}$ were found to be higher in the dry season than the wet season. This suggests that $As^{V}$ was more effectively reduced to $As^{III}$ in the dry season, possibly because of the lower pH environment found and relatively higher fraction of associated organic matter that is transported and deposited (less turbulence, restricted oxidation) and/or that greater oxidation/turbulence occurs in the wet season creating conditions for greater $As^{V}$

stability. It is noted that arsenic in the minerals from mines is usually in the lower oxidation state.

**Table 4-5 Arsenic species in surface sediment samples from the Pak Pa-Nang Estuary, total As=mean ± standard deviation (n=3).**

| | Dry Season 2001 | | | Wet Season 2002 | | |
|---|---|---|---|---|---|---|
| Sample | Total As ($\mu g\ g^{-1}$) | $As^{III}$ (%) | $As^{V}$(%) | Total As ($\mu g\ g^{-1}$) | $As^{III}$ (%) | $As^{V}$ (%) |
| ppn1 | 14.1±2.5 | 11 | 89 | 11.1±0.3 | 8 | 92 |
| ppn2 | 11.7±2.1 | 10 | 90 | 10.6±0.6 | 8 | 92 |
| ppn3 | 12.9±2.6 | 11 | 89 | 16.6±0.3 | 6 | 94 |
| ppn4 | 14±3.2 | 10 | 90 | 19.7±0.3 | 6 | 94 |
| ppn5 | 4.1±1 | 12 | 88 | 20.3±1 | 7 | 93 |
| ppn6 | 6.8±2.3 | 12 | 88 | 19.4±0.3 | 8 | 92 |
| ppn7 | 12±2.3 | 20 | 80 | 19.8±0.6 | 8 | 92 |
| ppn8 | 8.8±1.6 | 14 | 86 | 20±0.5 | 7 | 93 |
| ppn9 | 7.9±1.4 | 12 | 88 | 8.4±0.4 | 8 | 92 |

### 4.2.2 Arsenic in water and SPM samples from the Pak Pa-Nang Estuary

- **Dissolved arsenic**

Dissolved arsenic in the water samples ranged from 3.8 to 12 $\mu g\ l^{-1}$ and 1.1 to 3.7 $\mu g\ l^{-1}$ in the dry season and the wet season, respectively (Figure 4-13 and 4-14 and tabulated in full is presented in Appendix A). The concentrations were found to be slightly higher out of the river mouths, especially at stations ppn 2 and ppn 3.

Comparing the dry and wet seasons, higher arsenic concentrations were found in the dry season than the wet season; the result of the dissolved arsenic content being diluted by the increased river flow and greater dynamic mixing in the wet season. It may also be noted that the lower flushing rate and longer residue times for water bodies in the estuary during the dry season may result in an extended interaction between the water column and the sediment/SPM. The dissolved arsenic species in the Pak Pa-Nang Estuary are shown in Table 4-6.

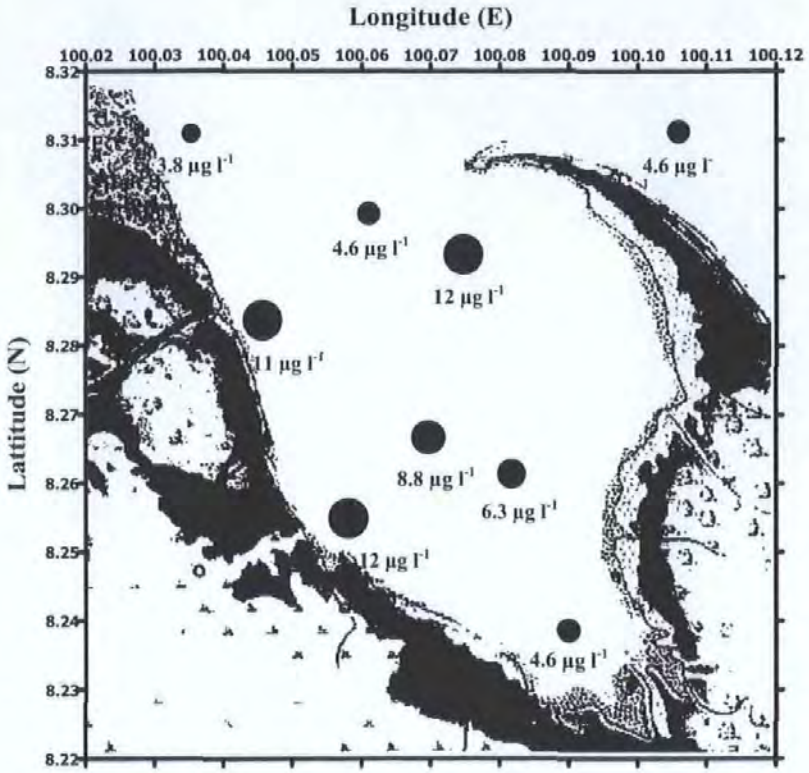



**Figure 4-13** Dissolved arsenic concentrations in water samples collected in dry season 2001 (n=3).

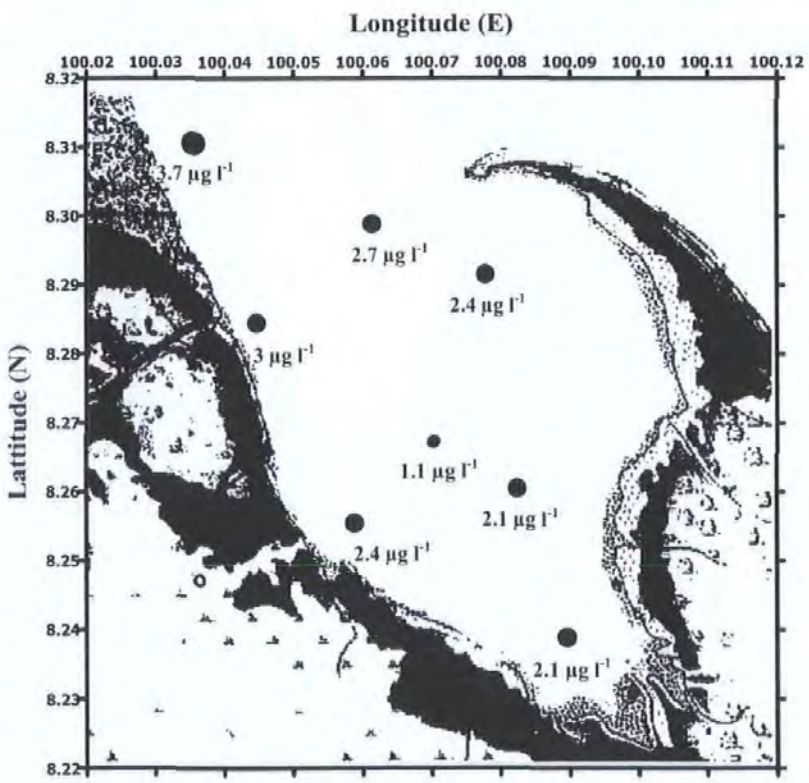



**Figure 4-14** Dissolved arsenic concentrations in water samples collected in wet season 2002 (n=3).

**Table 4-6 Dissolved arsenic species in the Pak Pa-Nang Estuary.**

| | Dry Season 2001 ($\mu g\ l^{-1}$) | | Wet Season 2002 ($\mu g\ l^{-1}$) | |
|---|---|---|---|---|
| Station | Total As | $As^{III}+As^{V}$ | Total As | $As^{III}+As^{V}$ |
| ppn1 | 4.6 | 4.6 | 2.1 | 1.3 |
| ppn2 | 12 | 7.1 | 2.4 | 0.7 |
| ppn3 | 11 | 6.3 | 3 | 2.1 |
| ppn4 | 5.4 | 5.4 | 3.7 | 2.9 |
| ppn5 | 4.6 | 3.8 | 2.7 | 2.1 |
| ppn6 | 8.8 | 5.4 | 1.1 | 0.9 |
| ppn7 | 6.3 | 5.4 | 2.1 | 2.1 |
| ppn8 | 12 | 7.9 | 2.4 | 1.9 |
| ppn9 | 4.6 | 2.9 | - | - |

Most of the dissolved arsenic was found in its inorganic form ($As^{III}$ and $As^{V}$) with values from 55 to 100% and 29 to 82%, in the dry season and wet season, respectively. In general the higher the concentration of total dissolved arsenic found, the higher the organic arsenic fraction and content. This was observed in the dry season, compared with the wet season and may be considered a result of the lower flows/longer residue of water bodies, organic content and increased reducing conditions.

- **Arsenic in SPM from the Pak Pa-Nang Estuary**

SPM samples filtered from water samples collected in the wet seasons of 2002 and 2003 were analysed for suspended particulate arsenic. The total SPM arsenic concentrations determined range from 3.8 to 6.8 $\mu g\ g^{-1}$ and 2.8 to 44 $\mu g\ g^{-1}$, respectively (Figure 4-15 and 4-16 and tabulated in full in Appendix A). Higher concentrations were found in 2003 than 2002, which may be attributed to the different rainfalls and specific timing of the sample collections. Higher rainfall could remobilize arsenic from the various main and intermediate sources and could be carried on SPM, especially on fine particulate matter, to the estuary. Since 'total' arsenic concentrations in the estuarine surface sediments were found to have a lower and narrower concentration range than that associated with the SPM, it would suggest that some of the arsenic carried by the SPM is flushed through and escapes to the Gulf of Thailand.

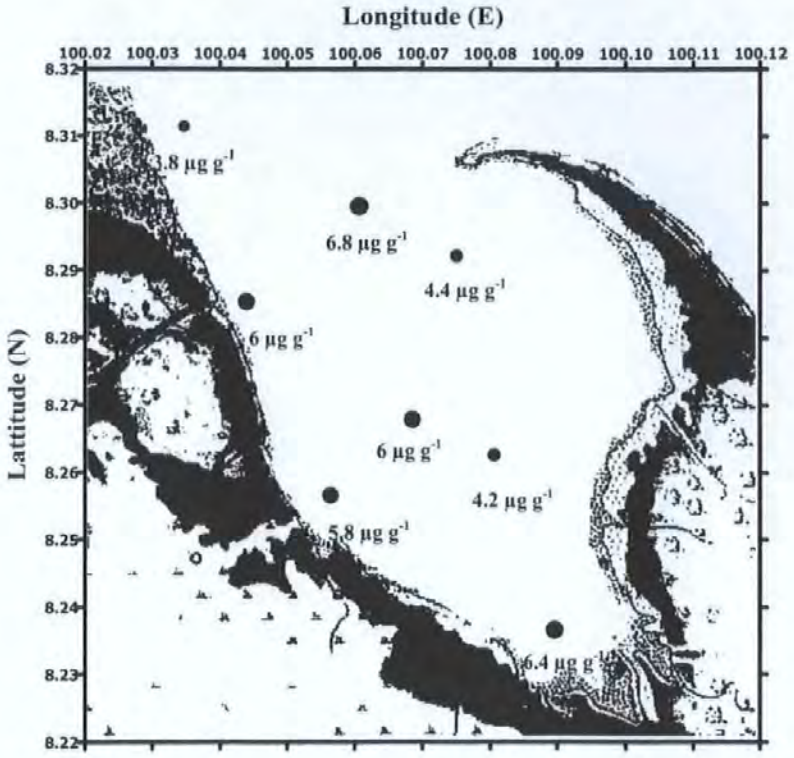



**Figure 4-15** **Distribution of arsenic in SPM samples collected in wet season 2002 (n=3).**

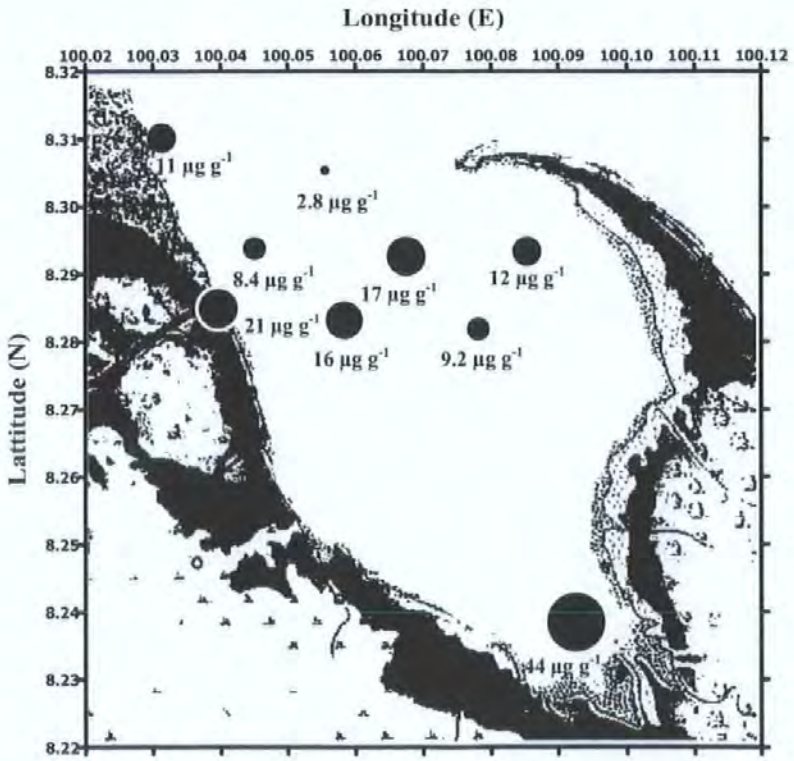



**Figure 4-16** **Distribution of arsenic in SPM samples collected in wet season 2003 (n=3).**

### 4.2.3 Seasonal variation of arsenic during wet and dry season

Seasonal exchange was also a factor of the cycle of arsenic in the Pak Pa-Nang Estuary. During periods of high winds in the wet season, dissolved arsenic concentrations are reduced to as little as 20% of dry season concentrations by sorption on to suspended sediments or incorporation into phytoplankton and precipitated to surface sediment. In the summer during dry season, arsenic is remobilized and returned to the water column, elevating the arsenic concentrations found (Figure 4-17).

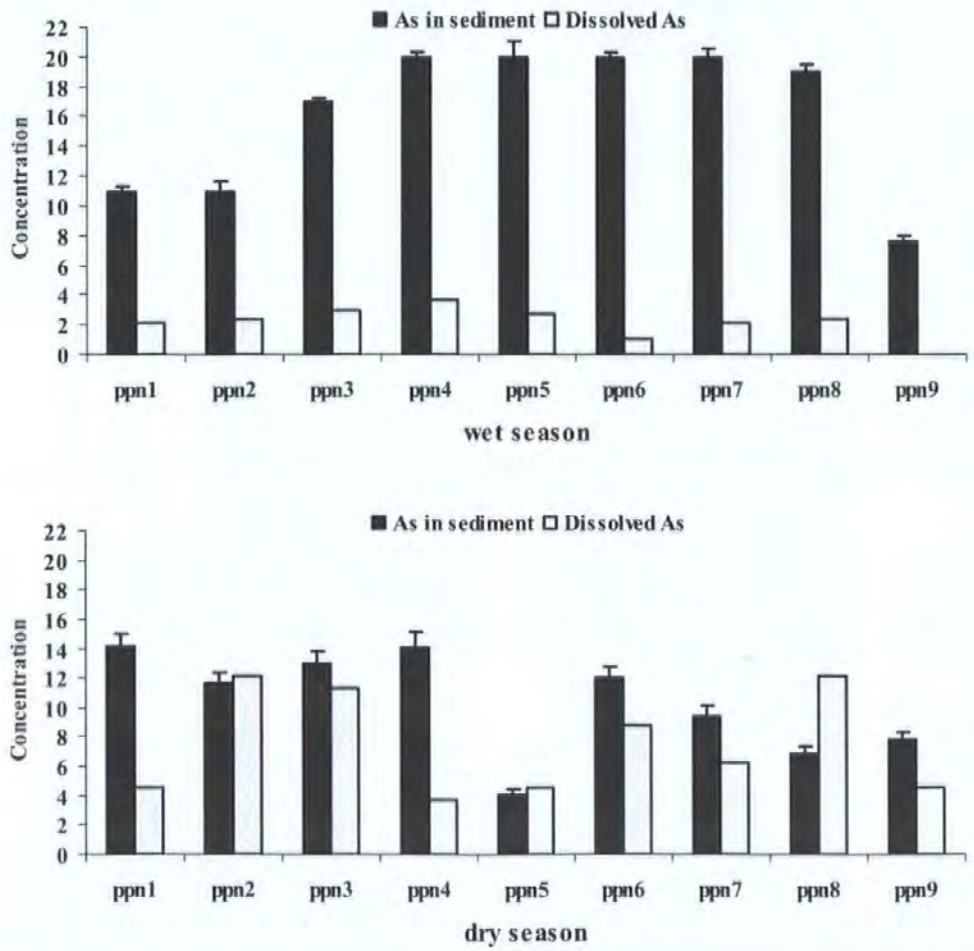



**Figure 4-17 Dissolved arsenic and arsenic in surface sediments of the Pak Pa-Nang Estuary, wet and dry season, the units of arsenic in sediment are in μg $g^{-1}$ and the units of dissolved arsenic are in μg $l^{-1}$.**

Belzile (1988)[10] analysed vertical profiles of arsenic in cores from the Laurentian trough in the Gulf of St Lawrence. The surface enrichment of solid arsenic and the increase of

dissolved arsenic with depth suggested that the mobile portion of arsenic is associated with iron oxyhydroxides. It follows a redox pattern of dissolution in the suboxic zone, upwards diffusion, and precipitation near the sediment-water interface under non-steady-state conditions.

Some micro-organisms such as a burrowing polychaete (*Nereis succinea*) has also affected the distribution and flux of arsenic from sediments by its production of irrigated burrows. These burrows increased both the effective surface area of the sediment and the diffusion of arsenic. Although physical suspension can produce large pulses of materials from contaminated sediments, it is the continuous biological activity that is likely to be more important in the mobilization of arsenic from sediments during dry season[11].

A tidal watch station (station 12 in Figure 3-7 Chapter 3) to monitor arsenic transport with time was set up in the Pak Pa-Nang Estuary during the 2003 sampling survey. Water samples were collected every 4 hours to determine arsenic-variation during the day. The samples were analysed for 'total' dissolved arsenic and total arsenic in SPM. The results (Figure 4-18) show a cycling of arsenic in SPM and dissolved arsenic some what out of place with the cycling of the tide. The arsenic concentration in SPM was elevated at low tide, where the riverine SPM could intrude into the estuary during low sea levels. Dissolved arsenic concentration was slightly elevated, where a higher concentration was found after the high tide. This lack of direct correlation between the tidal state and the arsenic concentrations could be due to the variable turbulence in the water column during the sampling period.

## 4.3 Arsenic species in fish and crustacea from the Pak Pa-Nang Estuary

The Pak Pa-Nang Estuary is well known as an important fishing ground. The primary goal of this section is to report the determination of concentrations of total arsenic and arsenic species in the edible tissues of Pak Pa-Nang fish, prawn and crab samples (features of those fish and shellfish samples are shown in Appendix B), and how these concentrations vary between different seafood. A secondary goal of this study is to provide arsenic speciation data to aid in the assessment of any human health-risk posed by arsenic in those samples[12].

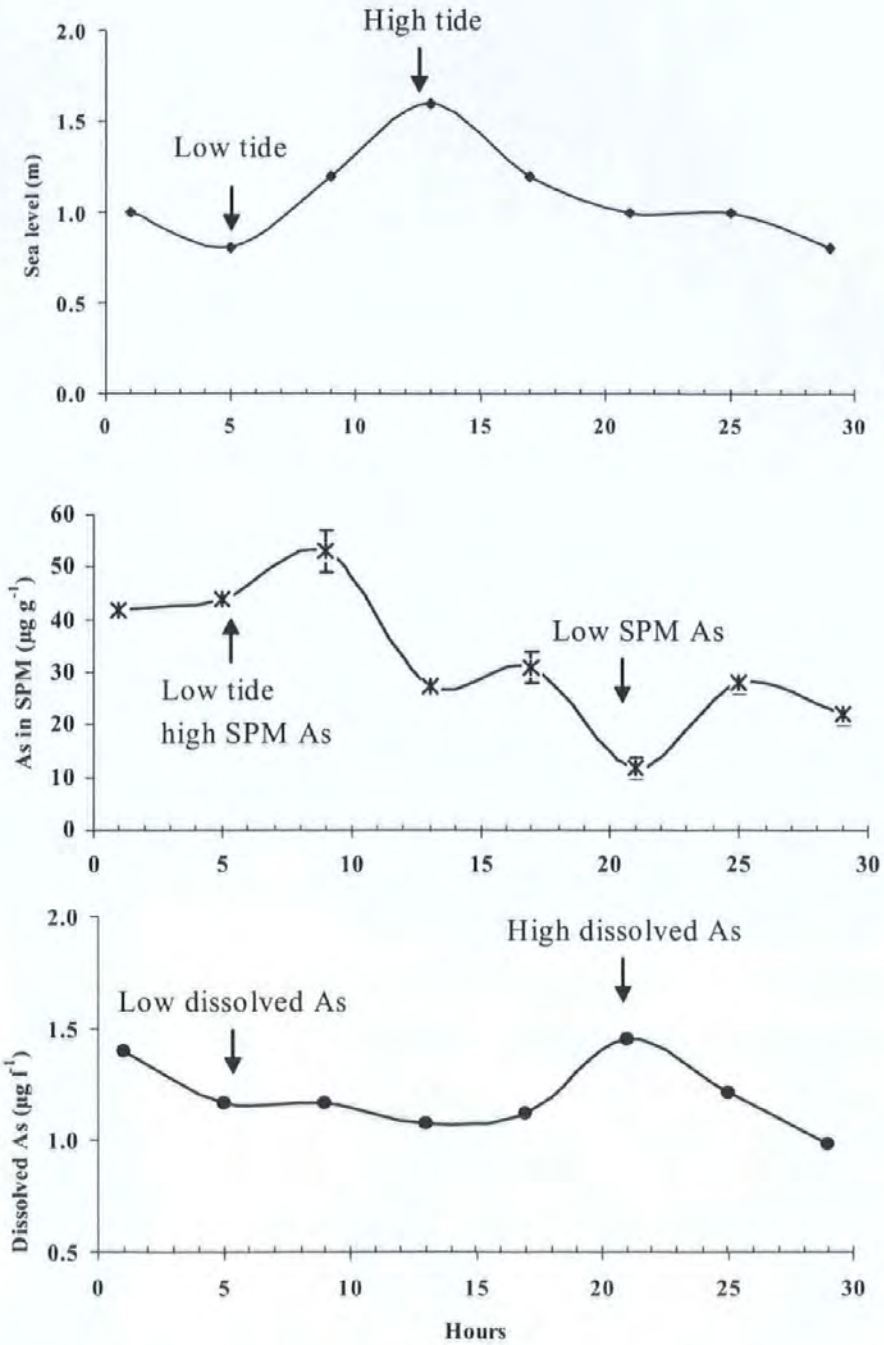



**Figure 4-18** **Arsenic in SPM and dissolved arsenic at the tidal watch station; Tidal level (units are in meters above Lowest Astronomical Tide) in the Pak Pa-Nang area (Pak Phun Hydrographic Station, 8° 32' N 100° 0' E).**

### 4.3.1 Arsenic mass balances from all biological sample analyses

Arsenic mass balance calculations were carried out on all biological samples where speciation analysis was performed. The arsenic species determined in each sample were summed. The total arsenic concentration in each sample should be equal to the enzyme extracted arsenic concentration. The mass balances for all biota are shown in Table 4-7.

**Table 4-7 Arsenic mass balance for the Pak Pa-Nang Estuary samples; mean ± standard deviation (n=3); dry weight basis.**

| Sample | 'Total' As [a] ($\mu g\ g^{-1}$) | Total As in extracts [b] ($\mu g\ g^{-1}$) | Sum of As species ($\mu g\ g^{-1}$) | Recovery (%) |
|---|---|---|---|---|
| **Fish** | | | | |
| Catfish (2001) | 2.6±0.18 | 2.5±0.3 | 2.5±0.28 | 96 |
| Catfish (2002) | 2.8±0.11 | 2.6±0.18 | 2.6±0.16 | 93 |
| Mullet | 2.9±0.11 | 2.8±0.19 | 3±0.43 | 97 |
| Croaker | 7.4±0.6 | 6.9±0.53 | 7.1±0.41 | 93 |
| Benthic fish | 7.9±0.31 | 8.2±0.42 | 8.4±0.58 | 104 |
| Sardines (2001) | 5.8±0.4 | 5.5±0.34 | 5.4±0.41 | 95 |
| Sardines (2002) | 5.3±0.16 | 5.3±0.45 | 5.5±0.42 | 100 |
| Leopard Scat | 2.6±0.15 | 2.5±0.33 | 2.7±0.39 | 96 |
| **Crabs** | | | | |
| Mud crab | 4.5±0.21 | 4.4±0.38 | 4.3±0.48 | 98 |
| Swimming crab | 17±1.1 | 16.8±0.69 | 17±0.66 | 99 |
| **Prawns** | | | | |
| Tiger prawn (Est.) | 11±0.48 | 11±0.49 | 11.2±0.63 | 100 |
| Tiger prawn (Farm) | 5.1±0.11 | 5.2±0.2 | 5.3±0.4 | 102 |
| Freshwater prawn | 5.2±0.22 | 5.7±0.32 | 6±0.45 | 110 |
| **Rice plant** | | | | |
| Rice root | 189±10 | 148±4.5 | 150±3.3 | 78 |
| Rice stem | 6.1±0.3 | 4.8±0.15 | 4.8±0.28 | 79 |
| Rice leaf | 4.6±0.14 | 4.0±0.16 | 3.9±0.22 | 87 |
| Rice grain 1 | 0.4±0.02 | 0.32±0.01 | 0.33±0.03 | 88 |
| Rice grain 2 | 0.4±0.03 | 0.34±0.02 | 0.32±0.02 | 85 |
| Cumin | 1.55±0.2 | 1.38±0.19 | 1.41±0.08 | 102 |

[a] Total arsenic using the acid microwave digestion, [b] Total arsenic using the enzyme extractions, trypsin for fish, crab and prawn and cellulase for rice, Est.=Estuary.

The results indicate that, in general, there is a good agreement between the total arsenic concentrations in the digests, and in the extracts and the sum of arsenic species in fish, crab and prawn samples. The exception is the rice plant and rice grains with the slightly lower values of arsenic in the extracts compared with those concentrations in the digests. The cellulase enzyme procedure, while 80% to nearly 90% efficient was not able to completely extract all the arsenic species from the rice plant and rice grain. Small amounts of arsenic were therefore recovered in the residues. While, it may be concluded that using trypsin enzyme extractions, for fish and shellfish is more efficient than using cellulase extraction for plants. The cellulose coating present in plant biota is robust and harsher extraction techniques tend to compromise the integrity of the species present.

### 4.3.2 Arsenic speciation in fish and shellfish

The total and inorganic arsenic concentrations measured in the fish and shellfish samples are summarized in Table 4-8. Concentrations of total arsenic ranged from 2.6 to 17 $\mu g\ g^{-1}$. The toxic species of primary concern is inorganic arsenic, which is a minor constituent. The general picture from the data is that the highest inorganic arsenic concentrations are found in the Croaker fish and the Swimming crab, both measured at 0.9 $\mu g\ g^{-1}$. The inorganic arsenic concentrations found in all these biota slightly exceeded the expected concentrations. It is not known wheter the elevated inorganic arsenic content found in some biota is present as the species in the tissues or present with the ultra-fine particulate matter trapped within the biota.

The arsenic species determined in the fish, crab and prawn samples from the 2002 to 2003 surveys are shown in Figure 4-19 and 4-20. In the fish and shellfish tissues from this study, arsenic is primarily in the organic form as arsenobetaine (ie. 69-82%). DMA is the minor species, where arsenic concentrations ranged from 6 to 19% of the total arsenic present. Inorganic arsenic accounted for some 5-13%, being less than 0.9 $\mu g\ g^{-1}$ in all samples.

In the tissue of the tiger prawns and swimming crabs from the estuary, which are benthic feeding crustacea, the concentrations of total arsenic ranged from, 11 to 17 $\mu g\ g^{-1}$. These values are higher than those in the tissues of fish (Table 4-7).

**Table 4-8 Arsenic concentrations in fish, crab and prawn samples from the Pak Pa-Nang Estuary, total As=mean ± standard deviation (n=3), dry weight basis.**

| | Species | Sampling period | Total arsenic ($\mu g\ g^{-1}$) | Inorganic arsenic ($\mu g\ g^{-1}$) |
|---|---|---|---|---|
| **Fish** | | | | |
| | Catfish *Plotosus canius* | August, 2001 | 2.6±0.18 | 0.2±0.01(8%) |
| | Catfish *Plotosus canius* | December, 2002 | 2.8±0.11 | 0.3(12%) |
| | Mullet *Mugil cephalus* | December, 2002 | 2.9±0.11 | 0.4(13%) |
| | Croaker *Johnius belangerii* | August, 2001 | 7.4±0.6 | 0.9±0.04(12%) |
| | Benthic fish | December, 2002 | 7.9±0.31 | 0.7(8%) |
| | Sardines *Escualosa thoracata* | August, 2001 | 5.8±0.4 | 0.3±0.01(5%) |
| | Sardines *Escualosa thoracata* | December, 2002 | 5.3±0.16 | 0.6(11%) |
| | Leopard Scat *Scatophagus argus* | December, 2002 | 2.6±0.15 | 0.3(11%) |
| **Crabs** | | | | |
| | Mud crab *Scylla serrata* | December, 2002 | 4.5±0.21 | 0.5(12%) |
| | Swimming crab *Portunus pelagicus* | August, 2001 | 17±1.1 | 0.9±0.03(5%) |
| **Prawns** | | | | |
| | Tiger prawn *Penaeus monodon* | December, 2002 | 11±0.48 | 0.8(7%) |
| | Freshwater prawn *Macrobrachium rosenbergii* | December, 2002 | 5.2±0.22 | 0.4(7%) |

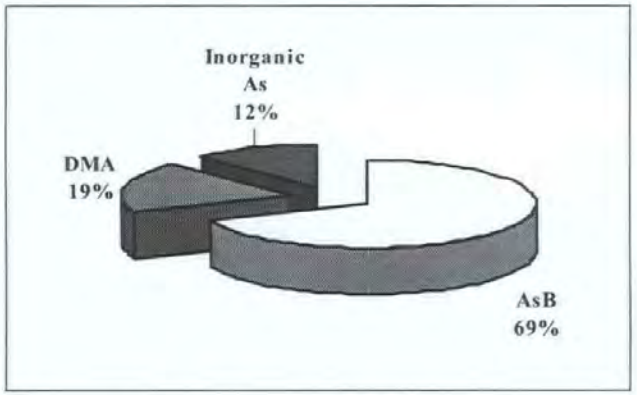
The tiger prawns from the estuary contained more than twice the amount of arsenic of those tiger prawns from the farm. It is also noted that the amount of total arsenic in the shell of farmed tiger prawn was 4.7±0.1 $\mu g\ g^{-1}$, similar to that of the tissue. The difference in the arsenic concentrations between natural and farmed tiger prawns may be related to the arsenic concentrations in the sediments. The sediments in the estuary had concentrations in the range of 10.4 to 16.2 $\mu g\ g^{-1}$, where the sediments of the farm

had a mean concentration of 8.4±0.4 μg $g^{-1}$. Therefore, the natural tiger prawns appeared to be affected by arsenic inputs to the estuary, and the farmed tiger prawns are fed on commercially available feed which may have a lower arsenic concentration. Arsenic speciation information for biota from the Pak Pa-Nang Estuary is extremely limited and with little published in the literature. However, the concentrations of 'total' arsenic found in this study agree well with the previous study by Boonchalermkit et al.[13]

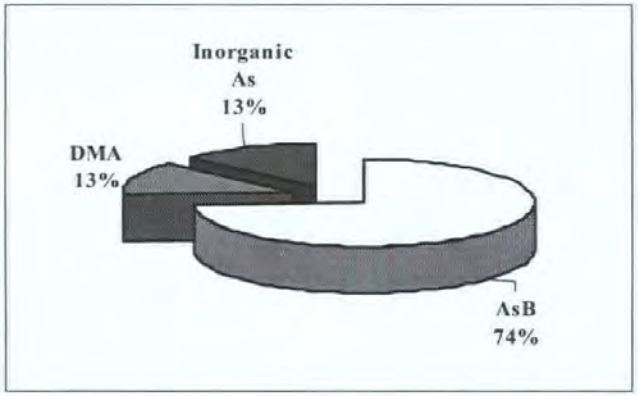
In this early study of total arsenic and limited qualitative arsenic speciation only in edible tissues of fish and shell fish samples collected from the Pak Pa-Nang Estuary. The samples studied were: five species of fish (*Liza vaigiensis*, *Arius truncatus*, *Plotosus anguillaris*, *Sillago maculate*, *Cynoglossus macrolepidotus*), Two species of shrimp(*Penaeus merguiensis*, *P. monodon*), one species of crab (*Scylla serrata*) and one species of green mussel (*Perna viridis*)[13]. In the tissue of pelagic feeding fish (*Liza vaigiensis*), total arsenic concentrations ranged from less than 0.002 up to 5.25 μg $g^{-1}$ dry weight, and in benthic feeding fish (*Arius truncatus*, *Plotosus anguillaris*, *Sillago maculate* and *Cynoglossus macrolepidotus*) the total arsenic concentrations ranged from 0.002 to 11 μg $g^{-1}$dry weight. The comparison was based on their habitats and life function. There is no significant difference in arsenic levels due to their different behaviour. However crab and shrimp tissues had higher levels of arsenic than the fish tissues, ranging from 0.38 to 32.3 and 0.11 to 12.8 μg $g^{-1}$ (dry weight), respectively.

Boonchalermkit et al.[13] also measured total arsenic in the green mussel, which remains stationary in the monitoring area, in order to study their arsenic accumulation in the estuary. The concentrations ranged from 0.32 to 25.2 μg $g^{-1}$dry weight (Figure 4-21).

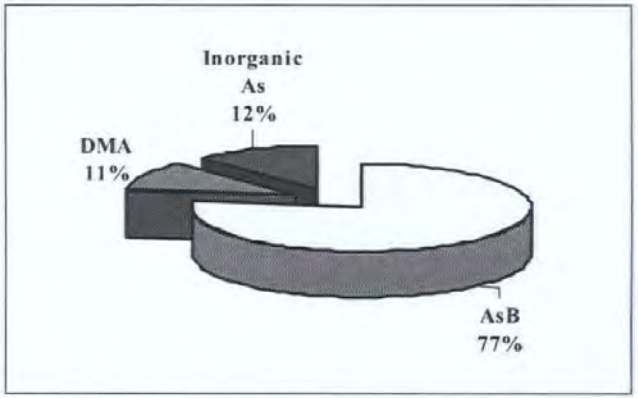
The trend shown in Figure 4-21 when compared with the surface sediment valued (Figure 4-10) and the SPM values (Figure 4-16) from this study, provide some insights into the transport of arsenic and its bioavailability in the estuary area and beyond. Since mussels are filter feeders, the arsenic accumulated in mussels seem to support the early stated hypothesis of the continuing, wide distribution of arsenic on a temporal basis and the inference that arsenic is escaping to the Gulf of Thailand in suspended form.



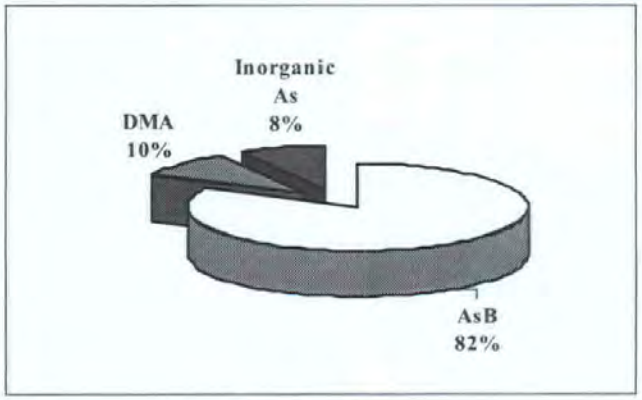

Catfish (*Plotosus canius*)



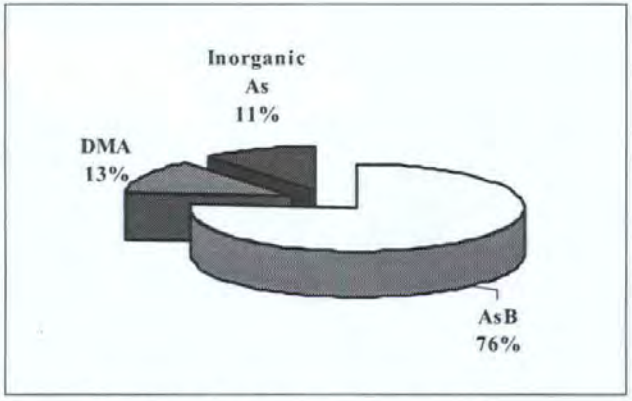

Mullet (*Mugil cephalus*)



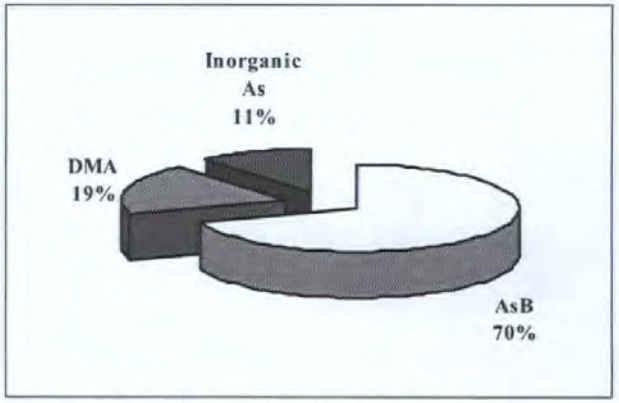

Croaker (*Johnius belangerii*)




Benthic fish

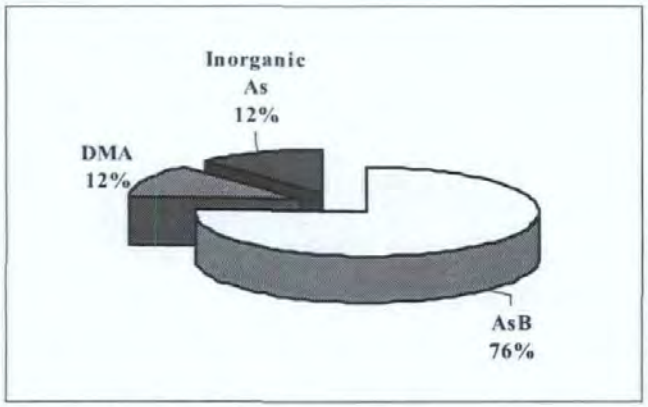



Sardines (*Escualosa thoracata*)

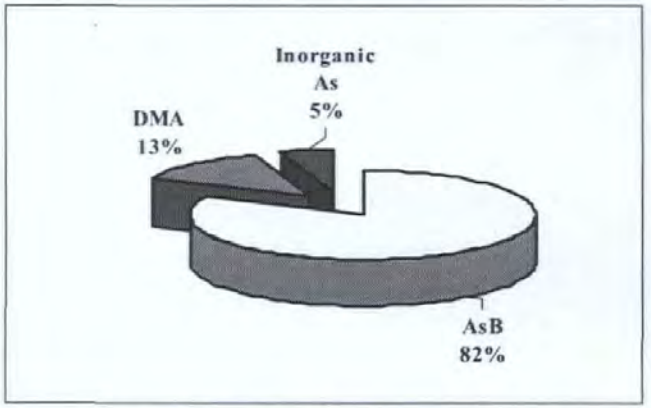



Leopard Scat (*Scatophagus argus*)

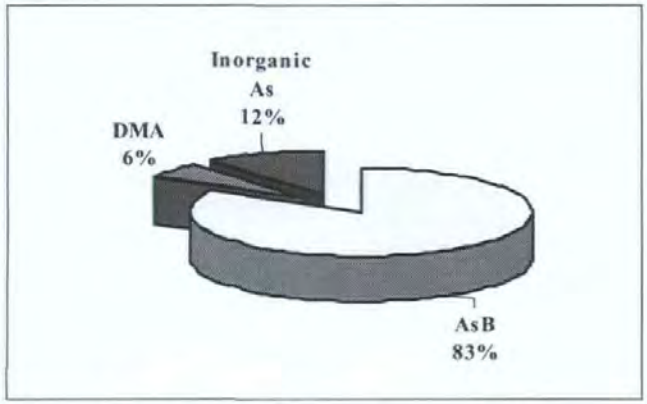
**<u>Figure 4-19</u> Arsenic species in fish samples from the Pak Pa-Nang Estuary; n=3 for each species; total arsenic in Catfish = 2.6±0.18 μg $g^{-1}$, Mullet = 2.9±0.11 μg $g^{-1}$, Croaker = 7.4±0.6 μg $g^{-1}$, Benthic fish = 7.9±0.31 μg $g^{-1}$, Sardines = 5.3±0.1 μg $g^{-1}$ and Leopard Scat = 2.6±0.15 μg $g^{-1}$.**



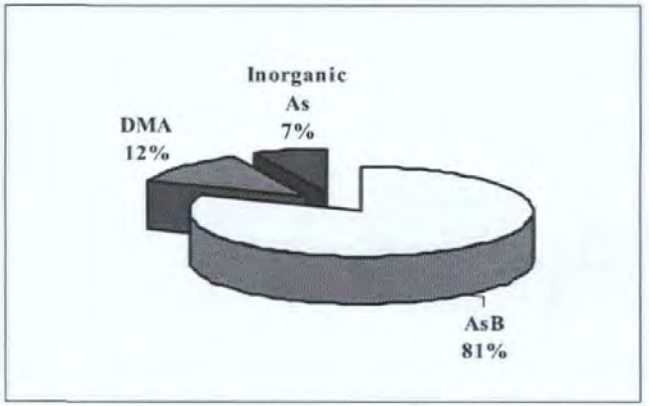

Mud crab (*Scylla serrata*)




Swimming crab (*Portunus pelagicus*)




Tiger prawn (*Penaeus monodon*)




Freshwater prawn (*Macrobrachium rosenbergii*)

**Figure 4-20 Arsenic species in crab and prawn samples from the Pak Pa-Nang Estuary; n=3 for each species; total arsenic in Mud crab = 4.5±0.21 μg $g^{-1}$, Swimming crab = 17±1.1 μg $g^{-1}$, Tiger prawn =11±0.48 μg $g^{-1}$ and Freshwater prawn =5.2±0.22 μg $g^{-1}$.**

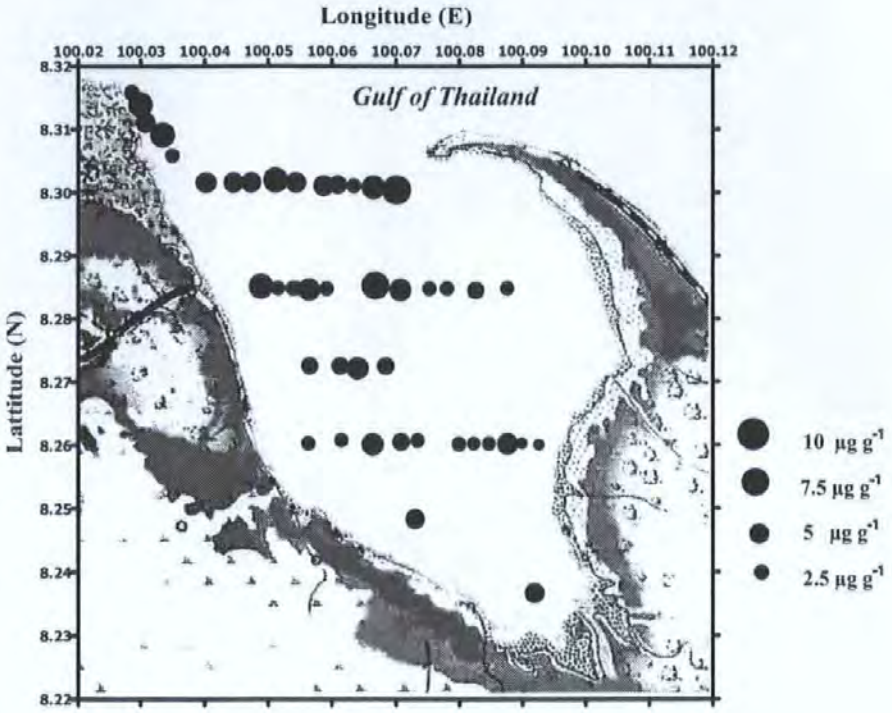



**Figure 4-21** **'Total' arsenic concentrations in mussel samples (dry weight basis) collected at aquaculture locations in the estuary by Boonchalermkit et al. [13] in November 1995 (wet season).**

## 4.4 Summary of the studies of total arsenic and arsenic species in the samples of the Pak Pa-Nang River Basin

Sampling surveys and laboratory analyses were performed to obtain the results of total arsenic and arsenic species in the Pak Pa-Nang River Basin samples of sediment, water, SPM and biota. Relatively high concentrations of arsenic were found in the samples collected from mining area because of the releasing of arsenic from the substantial deposits in the abandoned tin mines. The highest total dissolved arsenic was 148 $\mu g\ l^{-1}$, found in a main stream of the mining area. Arsenic in sediment was also found relatively high concentration in the mining area, the highest concentration was nearly 300 $\mu g\ g^{-1}$ (dry mass), found in the Namkhun Canal. As the speciation of available arsenic from sediments, waters and SPM is the most toxic arsenic forms ($As^{V}$ and $As^{III}$). The potential is noted for a continued supply of toxic arsenic from the tin mines to the rivers and the estuary from periodic flood-water transport, which would elevate the contribution from these species to local population and environment. The further discussion in some particulars and also a setting up of a conceptual model to understand the dynamics of arsenic transported in the study area will be performed in Chapter 5.

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# Chapter 5

# Processes affecting the transport of arsenic in the Pak Pa-Nang River Basin : Discussion, Conclusions and Future Work

This chapter contains a discussion of the distribution and transport of arsenic, in the dissolved and particulate phases, and the concentrations found in biological species in the Pak Pa-Nang River Basin. At the start of this discussion it is important to mention that estimates of the arsenic content of the Ronpiboon mineralization zone of 75,000 $m^2$ put the total amount of arsenopyrite at about 5,100 tons[1]. The presence of arsenopyrite gives rise to high arsenic concentrations in water and sediments, which have been mobilized within the area of the past mining activities, of varying intensity, over about 100 years[2]. It is not known how much of the arsenic load has been transported from the source region nor how much is being moved at the present day. Thus, Chapter 5 will involve an assessment of the potential impact of arsenic mobilisation from the source region and the storage in the sediments of the upper reaches of the river basin, including the role of particulate arsenic species. The transport processes, and the present day arsenic fluxes, must be understood to predict the impact of arsenic transported from the contaminated area to coastal waters of the Gulf of Thailand.

An important part of Chapter 5 is to define a new understanding of the dynamics of arsenic movement between the various compartments of the Pak Pa-Nang River Basin, by developing a conceptual model for arsenic transport. This could be of use in

improving the water management system. At the present time, this mainly satisfies the farmers' needs for water and no account is taken of the transport of arsenic throughout the River Basin.

## 5.1 Distributions, transport and storage of arsenic in the Pak Pa-Nang River Basin

The distributions of arsenic in its various forms and the processes that lead to the distributions are discussed in this section. These processes are important in the development of the conceptual model.

### 5.1.1 Behaviour of dissolved and suspended particulate arsenic

- **Dissolved arsenic**

Arsenic is transported in rivers and canals of the Pak Pa-Nang River Basin to the estuary as dissolved inorganic and organic arsenic. In this work, the inorganic form, comprising 55 to 85% of the total dissolved arsenic will be used in the calculations. Concentrations of dissolved inorganic arsenic in the Pak Pa-Nang River Basin and its estuary are plotted as a function of conductivity in Figure 5-1. Overall, the results showed that there were higher mean total dissolved arsenic concentrations detected in the river waters, 17 ± 39 $\mu g\ l^{-1}$ (n=21), compared with estuarine waters with mean concentration of 1.98 ± 0.96 $\mu g\ l^{-1}$ (n=24). However, differences in concentration occurred after rainfall. The river sampling in 2002 was carried out after the rains came and the mean dissolved arsenic concentration was 12 ± 32 $\mu g\ l^{-1}$, whereas the sampling in 2003 was conducted under relatively low flows with a mean dissolved inorganic arsenic concentration of 26 ± 46 $\mu g\ l^{-1}$. Thus, the high water flows caused dilution of the dissolved arsenic in 2002. In 2003, an elevated dissolved arsenic concentration of 148 $\mu g\ l^{-1}$ was detected in a main stream, in the mining area, which drains into the Namkhun Canal. The Namkhun Canal, which drains into the Pak Pa-Nang via the Kong and Kok Canals, had concentrations of dissolved arsenic in the range 66 to 122 $\mu g\ l^{-1}$. The general distribution of dissolved arsenic in the rivers was similar to the concentrations obtained in 1996 by Arrykul et al[3]. It must be stated, however, that the analytical quality assurance of their results was not given in detail and their data should be treated with caution.

In 2002, the mean dissolved inorganic arsenic concentration in the estuary was 1.75 ± 0.73 μg $l^{-1}$ and in 2003 it was lower at 0.92 ± 0.32 μg $l^{-1}$. One reason for the inter-annual difference in concentrations could be due to a dilution effect because of the greater freshwater flow in the wet season of 2003. Alternatively, the higher dissolved concentrations observed in the wet season of 2002, when the river flow was generally lower, could be due partly to fluxes of dissolved arsenic from the sediment porewaters, since dissolved arsenic in the porewaters of estuarine or coastal sediments can have concentrations a factor of 10 to 100 higher than in the water column[4,5].

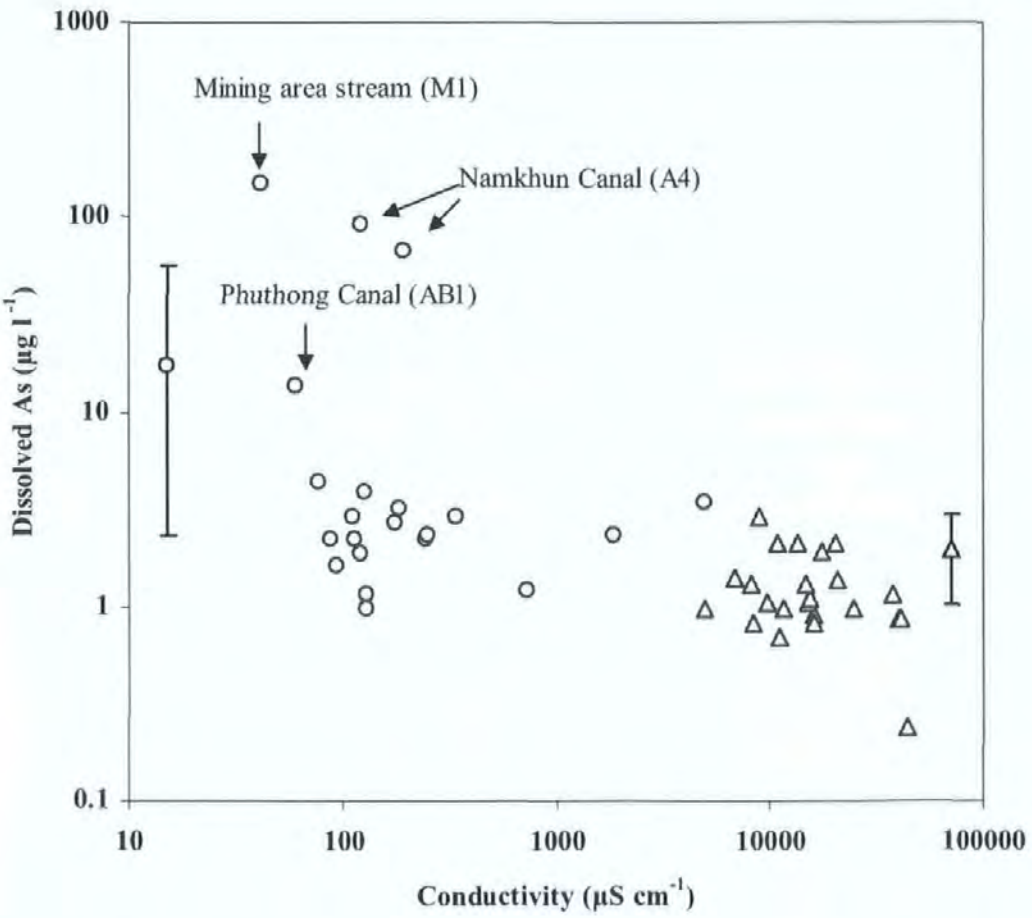


**Figure 5-1 Concentrations of dissolved inorganic arsenic in waters of the Pak Pa-Nang River Basin and its estuary; ○-riverine water [overall mean = 17.3±39 μg $l^{-1}$ (n=21)]; Δ-estuarine water [overall mean = 1.98±0.96 μg $l^{-1}$ (n=24)]. The measurements made near the source region are indicated.**

- **Dissolved arsenic - relationship with nutrients and chlorophyll**

An examination was made of the statistical relationships between the dissolved nutrients and chlorophyll (reported in Chapter 3) and dissolved arsenic. For the rivers there were no significant relationships, for $p<0.05$, between the dissolved nutrients and dissolved arsenic and between chlorophyll and dissolved arsenic, neither in the wet seasons (2002; 2003) nor the dry season (2001). Similarly, in the Bay there was no statistical relationship between dissolved phosphate and dissolved arsenic and between chlorophyll and dissolved arsenic in the wet and dry seasons. A relationship between dissolved arsenic and chlorophyll may have been anticipated if arsenic was being removed by phytoplankton. However, in studies of dissolved arsenic species in the North Sea a similar lack of relationship was observed[6]. This was ascribed to the different hydrodynamic behaviours of the dissolved and particulate phase, such that, because of settling, phytoplankton could be separated from the water parcel where they were removing dissolved arsenic.

However, the combined data in the Bay for the wet seasons of 2002 and 2003 gave a statistically significant relationship between the sum of the concentrations of dissolved nitrate and nitrite, defined as ($NO_3$+$NO_2$), and dissolved arsenic (Fig. 5-2a) ($R^2$=0.76; n=17; $p<0.001$) and between dissolved silicate and dissolved arsenic (Fig. 5-2b) ($R^2$=0.61; n=17; $p<0.01$). This implies that dissolved ($NO_3$+$NO_2$) and dissolved arsenic and the dissolved silicate and dissolved arsenic have a common source. The relationship with silicate is understandable in that arsenic is derived from the upper catchment where silicate is also weathered from the natural rocks (see Section 3.4.2). The association of dissolved arsenic with dissolved ($NO_3$+$NO_2$) is less clear because the latter has major sources in the coastal zone (see Section 3.4.2). However, the variability of the concentration of ($NO_3$+$NO_2$) in the rivers was relatively high because of inputs (e.g fertilisers) in the upper catchment. If the ($NO_3$+$NO_2$) inputs were in the vicinity of the mining area then close association between these two parameters was to be expected.

The differences between the concentrations in the dry and wet seasons are also shown in Figures 5-2a;b, although the linear relationships for the dry season are not significant. The intention is to illustrate the seasonal differences. The concentrations of dissolved ($NO_3$+$NO_2$) and silicate were significantly lower and in a narrower range in

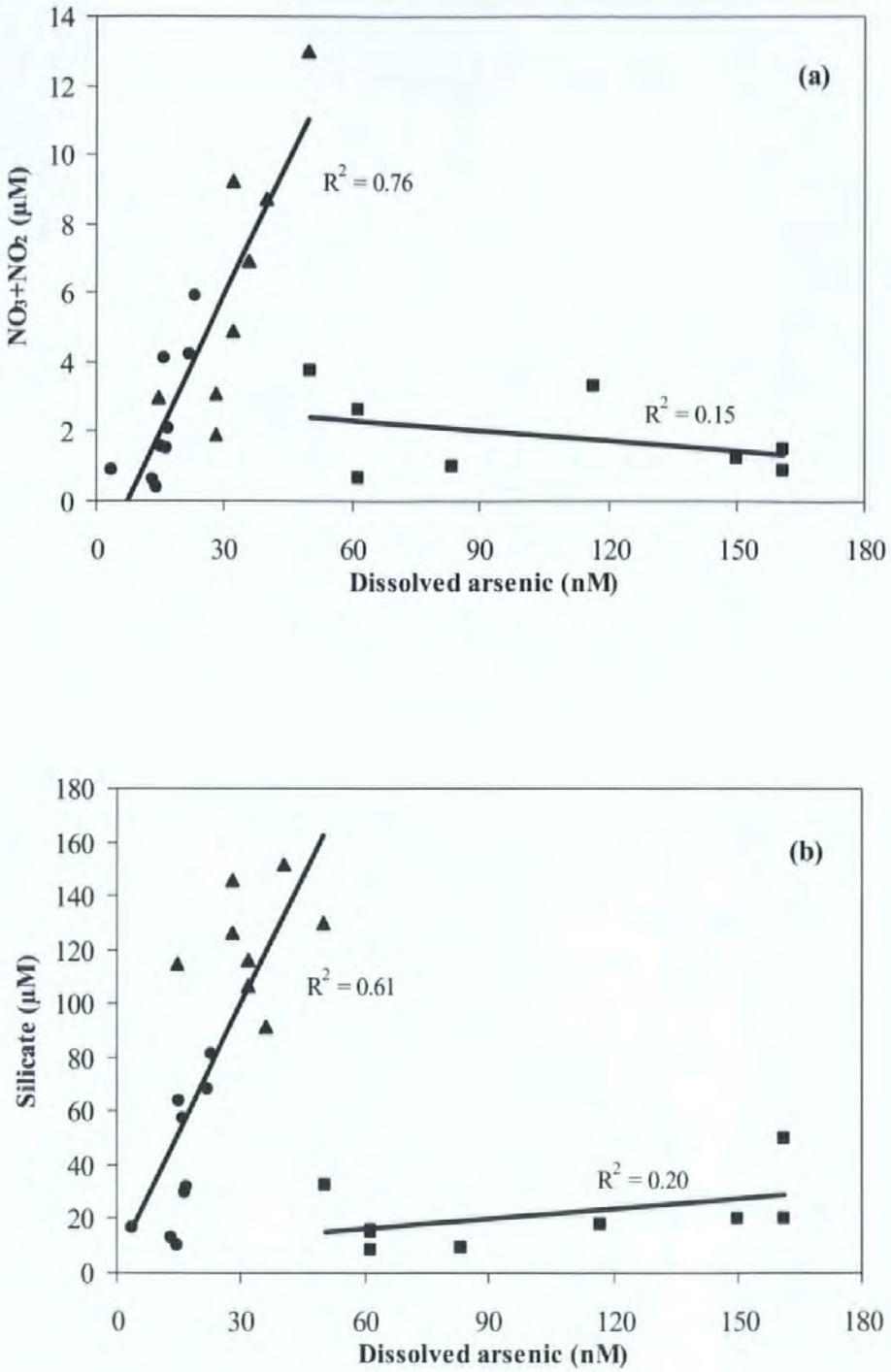


**Figure 5-2** **Concentrations of dissolved nutrients versus dissolved arsenic in Pak Pa-Nang Bay. (a) nitrate+nitrite and (b) silicate. ■ -August 2001 (dry season); ▲ -December 2002 (wet season); • -December 2003 (wet season). The regression coefficients for each line are given on the diagram.**

the dry season for the reasons given in Section 3.4.2. However, the concentrations of dissolved arsenic were generally higher than those in the wet season for three possible reasons; (a) the sampling was conducted just after low water, (b) river input was negligible as the barrier was closed and there was less potential for dilution and (b) dissolved arsenic inputs had occurred as a result of sediment-water exchange. It is not possible to identify which one is the main cause of the high dissolved arsenic concentrations and they may have all had a role in the elevated values obtained.

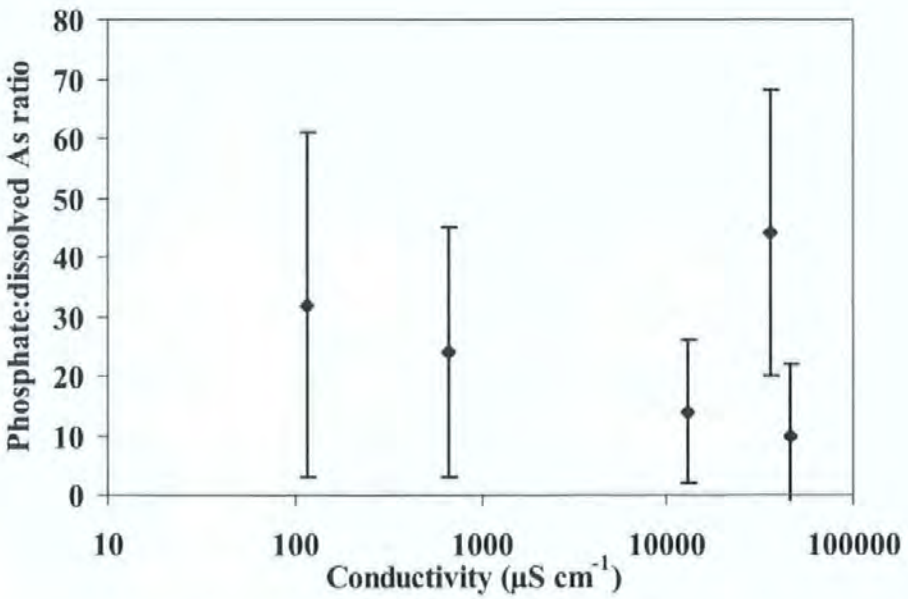


**Figure 5-3 The molar phosphate:dissolved arsenic ratio versus conductivity in the Pak Pa-Nang River and Pak Pa-Nang Bay.**

The mean molar ratio of dissolved phosphate to dissolved arsenic is potentially important in assessing whether arsenic (as arsenate) will be taken up by phytoplankton. The ratio in the rivers and the Bay was highly variable (Fig. 5-3) and there were no significant differences between the mean ratios of the sampling campaigns. The phosphate was always in excess over arsenic and raises the question as to whether dissolved arsenic was assimilated by the phytoplankton in the Pak Pa-Nang. In some cases phytoplankton are capable of

discriminating between arsenate and phosphate, although in other cases arsenate is removed with phosphate. It was not possible to determine whether uptake had occurred, by for example detecting the presence of dissolved methylated compounds, and this is an issue which will have to be addressed by workers in the future.

- **Particulate arsenic**

Concentrations of particulate arsenic versus conductivity are shown in Figure 5-4. In the rivers, the concentrations of particulate arsenic decreased rapidly as the conductivity increased. The mean concentrations of arsenic in SPM were 55 ± 100 μg $g^{-1}$ in 2002 and

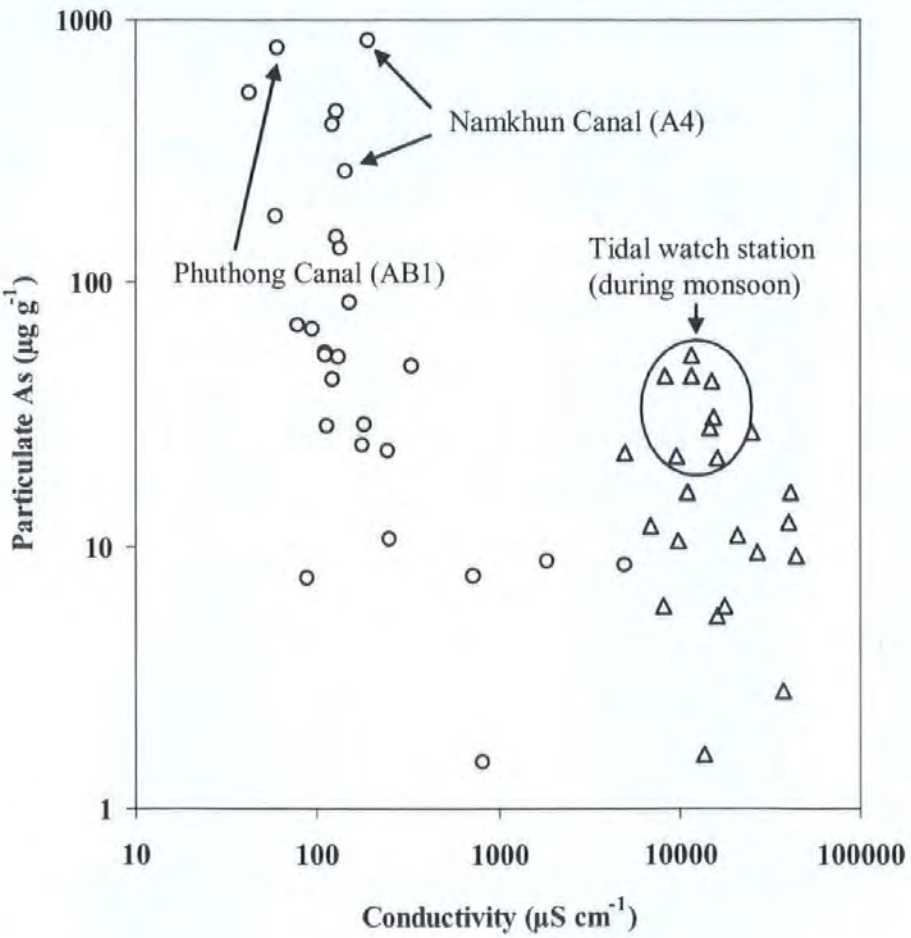


**Figure 5-4 Concentrations of particulate arsenic in waters of the Pak Pa-Nang River and its estuary; Combined data for 2002 and 2003. ○-riverine water; Δ-estuarine water. The measurements near the source region and at the mouth of the estuary are indicated.**

they were 277 ± 282 μg $g^{-1}$ in 2003, which demonstrates the effects of increased river flow in mobilising arsenic from the source area. The highest concentrations were found in the Namkhun and the Phuthong canals and the general distribution across the River Basin had a similar trend to that found in 1996[3]. The mean concentrations of particulate arsenic in the estuary were generally lower than those in the river basin, i.e. 7.8 ± 1.6 μg $g^{-1}$ in 2002 and 25 ± 15 μg $g^{-1}$ in 2003, as the arsenic-rich particulate matter from the rivers was diluted by uncontaminated SPM from offshore and possibly by phytoplankton. The concentration gradients of particulate arsenic were greatest in the western part of the estuary as they are carried in the plume of fresh water from the rivers. In the outer estuary, at the tidal watch station in 2003, higher concentrations of particulate arsenic were found compared with other times (Figure 5-4). This suggests that a fraction of SPM is made up of fine particles, with relatively high arsenic concentrations, which are permanently suspended in the water and have an ability to undergo long-range transport. This occurs particularly during the more turbulent conditions of the NE monsoon and it suggests that these particles by-pass the estuary and are transported directly into the Gulf of Thailand.

- **Particle-water interactions of arsenic**

The dissolved and particulate concentrations can be used to estimate the partition coefficients ($K_D$; defined as the ratio of the particulate arsenic concentration to the corresponding dissolved concentration) in the rivers and the estuary of the Pak Pa-Nang. Most data points have partition coefficients that lie between $10^3$ and $10^5$ l $kg^{-1}$ (Figure 5-5), which is a similar range to the values estimated for the Thames[5] and Humber[7]estuaries, UK and for the English Channel[8]. There is no obvious trend observed in the percentage of dissolved arsenic as a function of the SPM concentration, probably because of the variable input of arsenic from the contaminated zone and its reactions during transport to and within the estuary. Variation of the particulate phase concentrations of arsenic in the estuary could be due to intermittent supply from the river, although other factors may be important.

One of the other factors could be adsorption of the arsenate anion onto particles. This could be favoured in seawater because of the increases in the concentrations of major ions of seawater, such as $Ca^{2+}$ and $Mg^{2+}$. These may assist the uptake of arsenic onto

negatively charged natural particles[9] through complex formation (such as $CaH_2AsO_4^+$), if the behaviour of arsenate is assumed to be the same as that of phosphate[10].

Phytoplankton uptake has also been suggested as a possible removal process for dissolved arsenic in the water column[11, 12] and since there are high concentrations of chlorophyll in the Pak Pa-Nang Estuary this is another factor influencing the observed particulate concentrations.

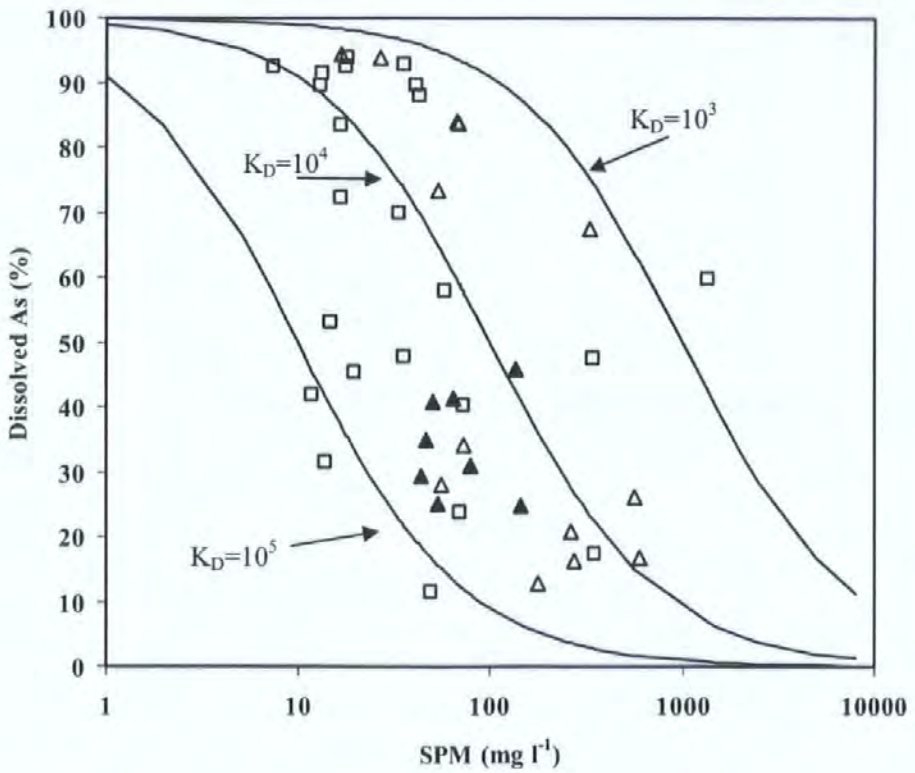


**Figure 5-5 Percentage of dissolved arsenic against SPM concentration in the Pak Pa-Nang River and its estuary; □-river water; Δ-estuarine water; ▲-estuarine water from the tidal watch station. The lines represent values of constant $K_D$ over the range of SPM concentration on the x-axis. The $K_D$s are identified on each curve.**

Normally, the fraction of the trace metals transported in the dissolved phase decreases as the SPM concentration declines, for example estuaries draining into the coastal waters of the North Sea, as described by Tappin et al[13]. In the case of the Pak Pa-Nang, the data in Figure 5-5 show the reverse of this trend, where the rivers generally had greater than 50% of the arsenic in the dissolved phase. Also, a significant number of the

samples from the estuary had less than 50% of the arsenic in the dissolved phase, particularly those samples collected at the tidal watch station. Thus, transport in the particulate phase appears to be an important factor in the estuary. This might be further evidence for a particle process, which specifically allows fine, arsenic-rich SPM to remain in suspension during its transport through the estuary.

### 5.1.2 Arsenic distributions in sediments and the storage of arsenic in the Pak Pa-Nang River Basin

The results in Chapter 4 showed that the arsenic contamination of river sediments differed between the four rivers surveyed during this work. The sediments of the Pak Nakorn and Pak Phaya canals appeared not to be as impacted by arsenic contamination as the Pak Pa-Nang River and the Bang Chak Canal. Thus, most of the discussion will be about the Pak Pa-Nang since it is also the largest river. The amount of arsenic in the sediments of the Pak Pa-Nang River Basin varies as a function of distance from the source region (Figure 5-6). The arsenic transported from the source region, by the various rivers and canals, will be governed by the different conditions in the high and low river flows. During dry season, arsenic has limited penetration down stream and can be stored in the upper river. If there are large amounts of arsenic in sediment stored in the upper regions of the rivers it may be remobilized during high flows.

An estimate of the inventory of arsenic stored in river sediments can be made using the data from the core at station A3. If the amount of arsenic in each slice of the core (Figure 4-3) is integrated over the depth of the core (i.e. 14 cm) then the total arsenic content is 9.1 $\mu g\ m\ g^{-1}$. If it is assumed that the density of the sedimentary material is 2000 $kg\ m^{-3}$, then the arsenic inventory for the A3 core is 18 g As $m^{-2}$. Assuming that the concentrations of arsenic in the sediments is uniform across the width of the estuary and along part of its axis, an approximate value for the total arsenic load can be obtained. The Pak Pa-Nang River is about 150 m wide at A3 and the storage of sedimentary arsenic may exist over several kilometres. These length values can be used to estimate the amount of arsenic stored in river sediments at A3, which amounts to 2.7 $t\ km^{-1}$, of which about 90% is as $As^V$, see Figure 4-4. The fraction of inorganic arsenic in the river sediments (see Figure 4-4) is similar to that found in soils, using a similar extraction method to that in this study[14]. This suggests that the sediments of the Pak Pa-Nang River contain an historical load of arsenic, mainly in the more toxic $As^V$ form.

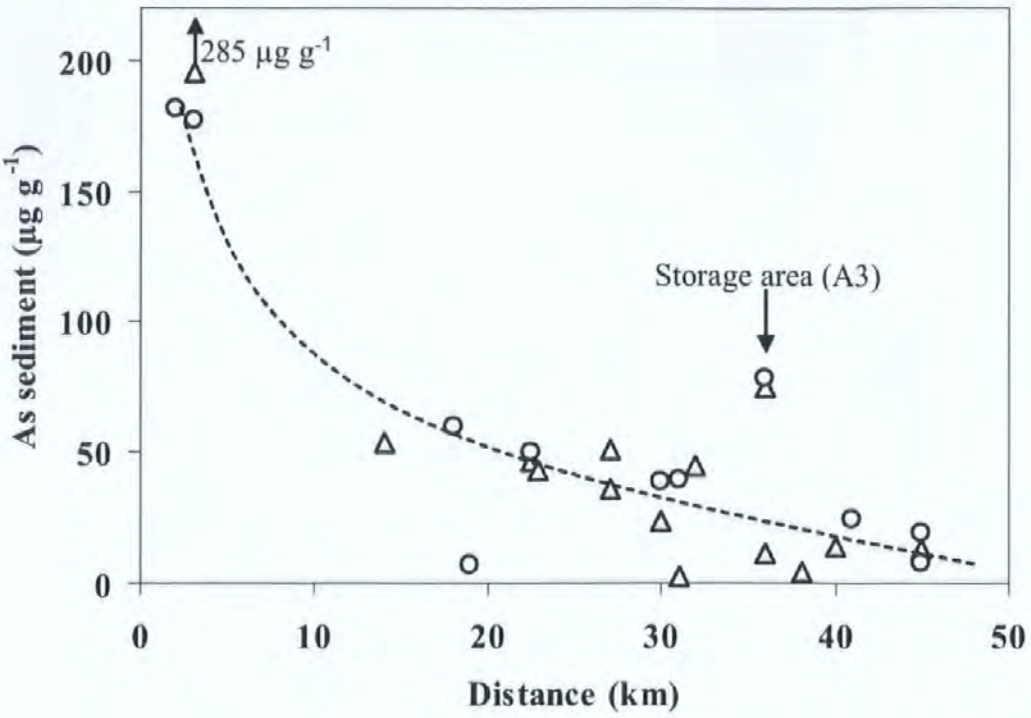


**Figure 5-6 Concentrations of arsenic in sediment versus distance from the source region in the Pak Pa-Nang River Basin; Δ-December 2002; ○-December 2003; The results for the Pak Nakorn and Pak Phaya are the lowest concentrations shown on the diagram; These sites are the least impacted by the arsenic contamination; The line is a visual estimate.**

Although the exact axial concentrations of arsenic in the Pak Pa-Nang near A3 are not known, the calculations suggest that there could be a significant amount of arsenic stored in these sediments. The extent to which this arsenic will be mobilised cannot be predicted with the limited amount of data from this study. However, climate change may cause changes in the river flows or the continued development of the canal system and changes in land use in the river basin may cause arsenic to be mobilised from the storage area and into the estuary, where it may impact the quality of seafood.

### 5.1.3 Fluxes of arsenic in the Pak Pa-Nang River Basin

- **River fluxes**

The Namkhun Canal directly flows from the mining area and transports a flux of arsenic from the mineralization zone further down stream[1]. Seasonal variations in the water

flows are dominated by variations in the rainfall and the only measurements in the Namkhun Canal were made by the Japan International Cooperation Agency[2]. The flux of dissolved and particulate arsenic was calculated by multiplying the arsenic concentration by the appropriate water flow. The results of the calculations are given in Table 5-1 and they show that the total mount of arsenic released daily from the source region was approximately 2 kg and 37 kg during low and high water flow, respectively. The relative proportion of arsenic transported in the dissolved and particulate phase varies seasonally and is a function of the concentration of SPM[5]. In the dry season approximately 56% of total arsenic was transported in dissolved phase, in wet season 65% is transported in suspended particulate phase when the river was more turbid and had a high suspended load.

**Table 5-1 Daily 'total' arsenic flux calculation in the Namkhun Canal during low and high water flow.**

| Season | Water flow | Particulate arsenic on SPM | | Dissolved arsenic | |
|---|---|---|---|---|---|
| | ($m^3$ $day^{-1}$) | As concentration ($\mu g\ l^{-1}$) | Flux (kg $day^{-1}$) | As concentration ($\mu g\ l^{-1}$) | Flux (kg $day^{-1}$) |
| Low flow | 8,640 | 836 x 0.12* | 0.87 | 122 | 1.1 |
| High flow | 138,240 | 263 x 0.66* | 24 | 93 | 13 |

*Particulate arsenic concentration and SPM concentration.

- **Estuarine fluxes**

Arsenic in the particulate phase plays an important role in the transport of arsenic in the Pak Pa-Nang Estuary. The particulate matter in the estuary is composed of various mineral phases derived from river transport. In addition, discrete and biogenic phases can be produced in situ through flocculation and biological activity, respectively. The total mean arsenic concentrations in surface sediment are 10.3 ± 3.5 and 16.2 ± 4.8 $\mu g\ g^{-1}$ in 2001 and 2002, respectively. This suggests that during the wet season a slight increase in sediment concentration occurred due to the increased riverflow, possibly bringing sediment contaminated with arsenic from the river storage regions. The particulate transport may happen intermittently throughout the wet period since high river flows, turbulent conditions, including high winds from the NE, can be interspersed with more quiet weather when deposition of arsenic-contaminated SPM can take place.

So some annual increase in the sediment arsenic load in the estuary might be expected during the wet season.

The total particulate arsenic concentrations in SPM from the Pak Pa-Nang Estuary, as a function of SPM concentrations, are shown in Figure 5-7. The mean concentrations in the sediments were similar to many of the values in SPM for 2002 and 2003 before the rains came. However, the sediment concentrations are significantly lower than the values in SPM found in the samples from tidal watch station in 2003, when conditions in the estuary were more turbulent. The tidal watch data in Figure 5-7 shows an increase in the concentration of particulate arsenic as the SPM concentration declines. This is a classical profile as described by Duinker[15], in which a metal is preferentially attached to permanently suspended particles, usually of small particle size and high in carbon (see Figure 3-12c), which provides good adsorption sites. This suggests that permanently suspended particles, which contains high concentrations of arsenic, remains in suspension and finally escapes the estuary into the Gulf of Thailand. The flux of particulate arsenic from the estuary to the Gulf cannot be estimated accurately at present because the residual flow is not known.

There are only very few reports of arsenic in the sediments of the Gulf but the sediments of the lower Gulf (about 200 km offshore)[16] have total arsenic concentrations of 5 $\mu g\ g^{-1}$. The rate of sedimentation in the central Gulf[17] is reported to be in the range 0.64 and 1.9 $kg\ m^{-2}\ a^{-1}$. Thus, if the arsenic-rich particles coming from the Pak Pa-Nang (mean $34 \pm 13\ \mu g\ g^{-1}$) make a significant contribution to the sedimentation, then the arsenic deposition in offshore sediments is between 22 and 65 $kg\ m^{-2}\ a^{-1}$. Whether the effect of the input of arsenic from the Pak Pa-Nang can be detected in the coastal waters with this amount of deposition can only be confirmed by further studies.

### 5.1.4 Impact of arsenic transport on marine biota

The mean concentrations of total arsenic in marine biota are generally less than 10 $\mu g\ g^{-1}$ and maximum values up to 17 $\mu g\ g^{-1}$ were obtained for swimming crab. The majority of the concentrations obtained in this study are similar to the range of concentrations of arsenic in a variety of marine species, for example, from the California coastal wetlands[18] and a Spanish estuary[19]. However, the concentrations for

crustacea in this study are lower than commercial species of crustacea from the Mediterranean Sea[20] and a recent paper also gives arsenic concentrations in fish, as wet weight, from the Mediterranean Sea[21]. Assuming that the dry:wet weight ratio is 0.25[22], then the arsenic concentrations given by Storelli and Marcotrigiano[21] are much higher than those found for fish in the Pak Pa-Nang. The concentrations of total arsenic in herring, cod and flounder from the North Sea were similar to those obtained in this study (after converting from wet weight to dry weight) and the total arsenic concentration was found to be directly related to salinity[23].

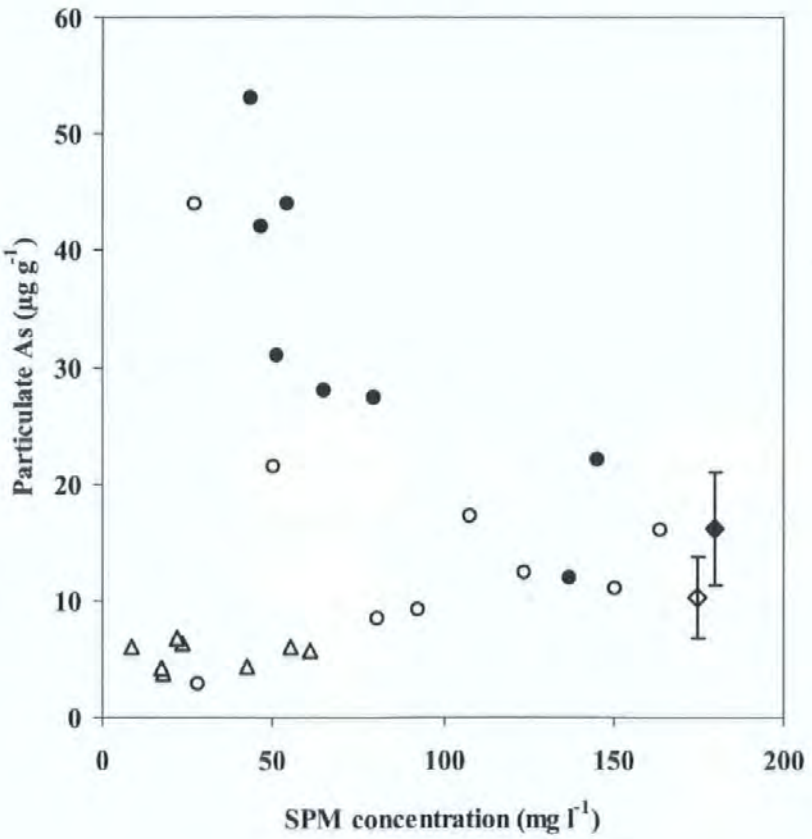


**Figure 5-7 Total particulate arsenic in SPM versus SPM concentration in the Pak Pa-Nang Estuary; Δ -December 2002; ○ -December 2003; • -December 2003 Tidal watch station; [◊ -total As in surface sediment, December 2002, mean = 10.3 ± 3.5 (n=9); ♦ -total As in surface sediment, December 2003, mean = 16.2 ± 4.8 (n=9)].**

During this study, in addition to the main organic arsenic fraction (e.g. AsB, MMA), fractions of the more toxic inorganic species were also found to be generally high in all the biota sampled. Figure 5-8 shows the proportion of inorganic arsenic, either as $As^{III}$,

$As^V$ or $As^{III}+As^V$ versus the total arsenic concentration for all the biota sampled. The mean inorganic arsenic fraction in marine biota is 11.5 ± 3.7%, with a coefficient of variation of 32%, which is a rather narrow range considering the variety of organisms sampled. However, the fraction of inorganic arsenic is relatively high when compared with results from other studies where a significant fraction of the arsenic was in the form of arsenobetaine[24-26]. There are exceptions to this general observation. For example, Branch et al[27] conducted a study of arsenic speciation in several different fish, where the proportion of inorganic arsenic that they found for mackerel, cod and dab is comparable to data from this study (Figure 5-8). Also, commercial species of crustaceans can have 3.5 to 5.7% of the total arsenic as inorganic arsenic[20] and inorganic arsenic was detected in fauna from the Guadalquivir Estuary in Spain[19]. These findings might also be of relevance to future studies of biota from the freshwater reaches of the Pak Pa-Nang since it has been shown that freshwater crustaceans can have between 18 and 34% of inorganic arsenic[28].

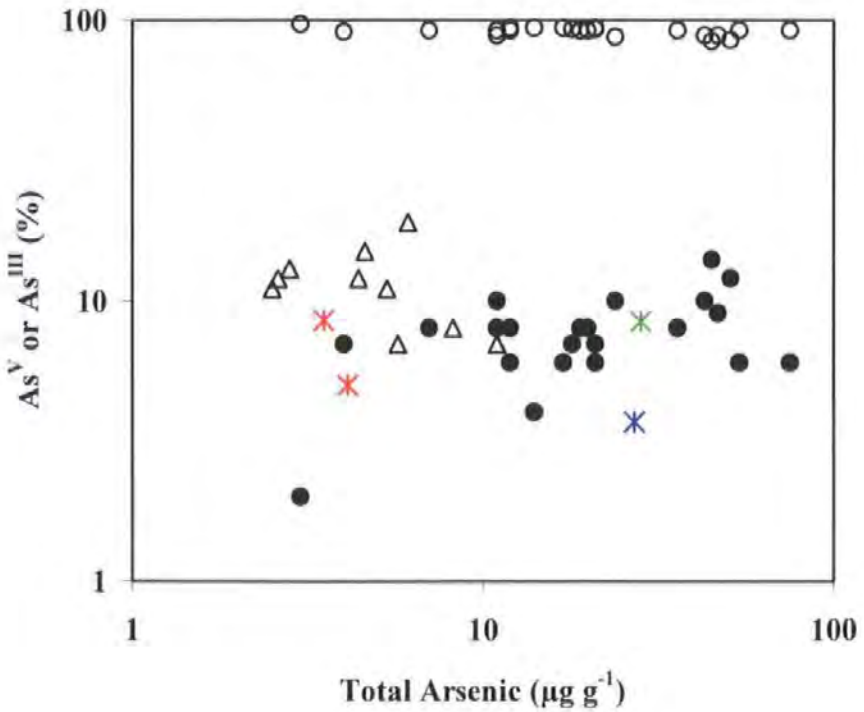


**Figure 5-8 Plot of the percentage of As species in SPM and in marine biota from the Pak Pa-Nang Estuary. ○ -$As^V$ in SPM; ● -$As^{III}$ in SPM; Δ -Inorganic As in marine biota from this study; Inorganic As in ✳ -Mackerel, ✳ -Cod, ✳ -Dab (Branch et al., 1994)[27].**

One reason for the high proportion of inorganic arsenic in the marine biota from the Pak Pa-Nang is the fact that some of the food sources in the estuary may contain high levels of inorganic arsenic. Figure 5-6 also shows the percentage of $As^{III}$ and $As^{V}$ in SPM from the estuary. Typically more than 90% of the arsenic is as $As^{V}$, with a few percent as the highly toxic $As^{III}$. If the estuary is receiving a continuous supply of particles with high levels of inorganic arsenic then this may by taken up by the marine biota and is reflected in the arsenic content of the tissue.

## 5.2 Conceptual model of arsenic transport in the Pak Pa-Nang River Basin

To summarise the field results, a conceptual model of arsenic transportation in the Pak Pa-Nang River Basin was developed (Figure 5-9). This was based on an existing approach applied to the Tamar Estuary[29], which was modified for application to the Pak Pa-Nang. The model was made up of four compartments, i.e. source region, river, estuary and gulf. The fluxes of arsenic between the compartments were assumed to be in the dissolved and particulate phases. It was also assumed, from the results, that arsenic in river and estuarine sediments may be eroded or deposited as the flow conditions increase or decrease, respectively. The fluxes of arsenic from the source region will depend upon the season, such that during the NE monsoon the high river flows will carry a greater amount of arsenic than in the dry season. The magnitude of the changes is reflected in the sizes of the arrows connecting the compartments. The possibility of sediment-water exchanges of arsenic in the estuary were introduced into the model, even though they were not measured in this study. The operation of the model is discussed as follows.

During low flow, in the dry season, deposition may take place in the upper river storage areas because of the low current speed and low turbulence. Arsenic accumulation in the river sediments is a dominant factor during dry season, although a smaller amount of particulate matter may pass from the rivers to the estuary and may deposit in the estuarine sediments. Because, in the dry season, the water and sediment movements in the estuary are mainly governed by the tidal conditions (the range is normally <1 m), the total amounts of dissolved and particulate arsenic escaping to the Gulf are potentially very low. Since the sediment columns are more stable at low flow (the sediment may be gently oscillating on the sea bed under the influence of the tide) it is possible that low oxygen conditions may develop in the sediments and diagenesis may

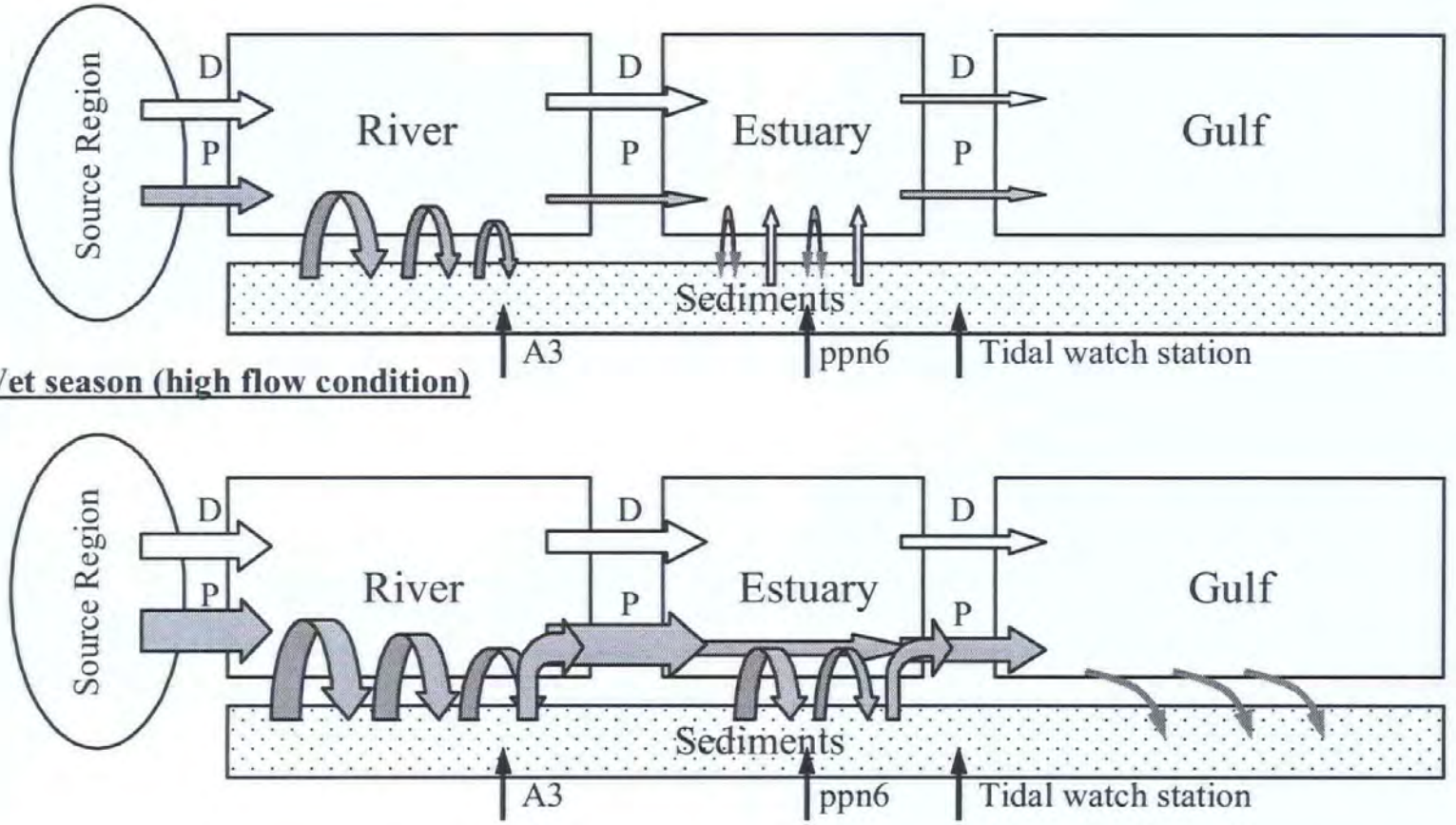


**Figure 5-9** **Schematic of arsenic transport in the Pak Pa-Nang River Basin and its estuary (modified from Morris et al. 1986); D -Dissolved arsenic (open arrows); P -Particulate arsenic (shaded arrows). The thickness of the arrows indicates the general intensity of the flux. In the dry season a sediment-water flux of dissolved arsenic is indicated.**

release arsenic from the particles. The arsenic in the porewaters may diffuse into the shallow water column, assisted by the regular stress of tidal shear, causing an increase in the dissolved arsenic concentrations (i.e. compared with the wet season). This hypothesis needs to be tested by taking samples of the porewaters. In the wet season, the higher water flows carry a greater amount of particulate arsenic from the source area, through the rivers to the estuary. The amount of arsenic-rich material coming from the catchment is increased by the erosion of sediments (deposited in the dry period) in the storage region of the rivers. The particulate flux increases significantly in the wet period as shown in Figure 5-9.

During the monsoon period the estuary is affected by strong NE winds and wave actions and increased freshwater runoff. Thus, some of the material coming from the rivers will have a relatively high settling velocity and may deposit in the estuary. The SPM with low settling velocity and in permanent suspension will be carried out to the Gulf during the ebbing tide. As mentioned in Section 5.1.3, the SPM transported into the coastal region may ultimately deposit in near shore sediments creating a potential health hazard to bottom dwelling organisms.

## 5.3 Impacts on environmental and human health

Some of the concentrations of arsenic in the various environmental compartments that have been tested in this study appear to be low and close to background values[30]. However, a recent assessment has been conducted by the Oslo and Paris Commissions (OSPAR) of acceptable values for Environmental Assessment Criteria (EAC) (previously called Ecotoxicological Assessment Criteria), which are expressions of biological and/or ecotoxicological risk[31]. The OSPAR definition of an EAC is "the concentration below which no harm is expected" and "above which concern is indicated". An established or firm EAC is used to compare with field data to provide information on whether a contaminant is present in the environment at concentrations high enough to lead to adverse biological effects, potentially.

The recent OSPAR assessment[31] has led to a firm definition of the range of concentrations for dissolved arsenic of 1 to 10 $\mu g\ l^{-1}$ and a provisional definition of the range of concentrations for arsenic in sediments of 1 to 10 $\mu g\ g^{-1}$. The use of a range allows a "traffic light" system to be used, such that field measurements of arsenic

concentrations that (a) are at or below the lower value and "should not give rise to any adverse biological effects" are given the Green Light, (b) are between the lower and upper values and "may give rise to possible biological effects (e.g. impaired growth; reproduction)" are given the Amber Light and (c) are above the upper value and "long-term biological effects likely (e.g. impaired growth, reproduction) and acute biological effects (e.g. mortality) possible" are given the Red Light.

Most of the dissolved arsenic concentrations in the Pak Pa-Nang Estuary, at any time of year, signal an amber light, whereas most of the sediments signal a red light at all times. Although the OSPAR EACs apply only to the marine environment, extrapolating them to the Pak Pa-Nang River Basin indicates that the red signal is very strong and that urgent action is required. As an indication of the potential effects of the long-term contamination on marine biota a study of the invertebrate macrofauna in the Fal Estuary (United Kingdom) has been carried out[32]. Like the Pak Pa-Nang, the Fal has suffered from arsenic and other heavy metal contamination over many years. It was found that certain crustaceans were completely absent from the Fal, when compared with 40 other locations in the UK in six separate estuaries. This finding was described as being due to the sensitivity of crustaceans to metal pollution, rather than any organic pollution. Whether the arsenic, or any other heavy metal[33], contamination has caused any changes in the composition of the marine biota in the Pak Pa-Nang Estuary and the coastal waters of the Gulf cannot be predicted without further study.

Although this study did not focus on the impact on human health the measurements of arsenic concentrations in the catchment give cause for concern. Since 1987 attempts have been made to improve the situation by piping water to the Ronpiboon region from deep bore holes in the mountains[34, 35] and by undertaking trials in phytoremediation of the contaminated areas[36, 37]. During this study, in a village house in the mining area a value of 30 $\mu g\ l^{-1}$ was determined in the drinking water, which was being piped from a newly dug bore-hole in the mountains, near the mine. This value is three times higher than that recommended by the WHO[38] and this drinking water is of poor quality. Also, people were still using the water to wash their pots and pans and cooking utensils[39] and in many places, the contaminated waters, e.g. the Namkhun Canal, were being used to wash clothes and to irrigate paddy fields and other small vegetable plots[40]. It is apparent that, despite a number of official studies and remedial actions (such as the piping in of

"clean water"), the situation is not improving and the number of arsenic-induced cancer cases continues to rise[41].

## 5.4 Conclusions

This study had two main themes (a) the development of analytical methods for determining total arsenic and arsenic species and (b) the application of these analytical techniques to the study of an arsenic contaminated area of southern Thailand.

- **Analytical Methods**

The analytical developments involved (a) optimisation of the sample digestion technique for total arsenic, (b) arsenic species extraction and separation-detection using HPLC-ICP-MS, (c) environmental sampling of sediment, water and biota in the vicinity of the Pak Pa-Nang River Basin and determination using the developed analytical methods. The development of a viable analytical method[42] allowed both the total arsenic concentrations and those of arsenic species, i.e. inorganic $As^{V}$, $As^{III}$, organic arsenic AsB, DMA, and MMA to be determined accurately. Trypsin was used in an arsenic extraction method that gave a high extraction efficiency and avoided arsenic species degrading during extraction. It was used to extract arsenic species from freeze dried fish and shellfish tissue in a slightly alkaline environment, about pH 8 at 37 $^{\circ}C$. The choice of a phosphoric acid extraction for arsenic species in sediment was following Thomas et al. (1997)[43] and Gallardo et al. (2001)[44] who demonstrated the successful extraction earlier and Garcia-Manyes et al[14] who recently applied this extraction method to soils.

- **Land-ocean transport of arsenic**

The use of arsenic-contaminated water is a major cause of disease found in the Ronpiboon mining area, where people use the water in the irrigation canals and rivers for drinking, food preparation and washing. Analysis of arsenic concentrations in sediments and waters revealed the transport of arsenic from the source region in the catchment and its spread via the estuary, rivers and canals. The arsenic flux increased considerably during the wet season, November to January. Two canals in the river basin, Namkhun and Phuthong were found to have a concentration of dissolved arsenic above the safety limit of 10 $\mu g\ l^{-1}$. The study found that considerable quantities of

arsenic (i.e. kilogrammes per day) were entering the river system from the source area during the monsoon season. In the Pak Pa-Nang River Basin elevated arsenic concentrations were found in surface sediments and in sediment cores. Storage of high arsenic concentrations in the sediment, possibly as much as 2.7 t $km^{-1}$, may affect the river environment for many years, particularly since toxic inorganic arsenic was found to be the major species in the sediments.

Arsenic has been transported to the estuary via rivers mostly in suspended form. Arsenic analysis of the sediments revealed an average concentration range from 4-20 μg $g^{-1}$, with a decreasing concentration gradient outwards from the four river mouths in the dry season. In the wet season, the gradient of higher arsenic concentrations was moved towards the centre of the estuary and this may be a consequence of reduced water dynamics and/or flocculation processes through the mixing of river water and seawater at the centre of the estuary. Dissolved arsenic concentrations in the estuary ranged from 1.1-12 μg $l^{-1}$ and higher concentrations were found in the dry season compared with the wet season as a result of dilution by the increased river flow in the wet season and possibly sediment-water exchange of dissolved arsenic during the dry season. Although the concentrations of dissolved and sedimental arsenic are relatively low in the estuary, in many cases they exceed the upper threshold of the established EACs. Attention should be paid to reducing the concentrations to acceptable levels, particularly since the impact on biological species abundance and richness is not known for the Pak Pa-Nang.

Water management plays an important role for arsenic distribution to the wider area of the Pak Pa-Nang. A model has been developed to explain more clearly the processes that are affecting arsenic transport in the River Basin. At the moment no account is taken of the threat from arsenic transport and this should become part of the water management system in the future. A better water management system will be essential if there are any changes in the rainfall due to climate change and if there are changes to land use in the catchment. It will be important to take account of these changes so that the spread of contamination is limited.

Water quality monitoring in the estuary has also drawn attention to the fact that other contaminants may also cause environmental degradation in the estuary, such as the discharge of high concentrations of nitrogen compounds from shrimp farms and uncontrolled community sewage.

- **Arsenic in foodstuffs**

The determination of arsenic in a rice plant revealed that the roots of the rice plant accumulate the greatest level of arsenic, followed by the stem, the leaf and the rice grain. MMA was found as the major species with a lower amount of inorganic arsenic in the rice root, stem and leaf. The proportion of inorganic arsenic declined from root, stem and leaf and only MMA was found in rice seed. Irrigation of the paddy fields with water from the source region may be a common practice. However, since access to the paddy fields and identifying the source of the irrigation water is not easy a much more comprehensive survey is required before conclusions can be drawn about the impact of water with high arsenic concentrations on rice growing. The study did find elevated arsenic concentrations in the stem and the leaves of rice plants, which are often used for feed or bedding for domestic animals.

Many small-scale fishermen make their living from fishing in the estuary and arsenic contamination has been a factor of concern. The total arsenic concentrations in the fish and shellfish samples from the estuary were relatively low (not greater than 20 $\mu g\ g^{-1}$). The major species present and available in fish and shellfish was the non-toxic form, arsenobetaine (69 to 83%), with smaller quantities of the mildly toxic DMA (6 to 19%). The highly toxic inorganic arsenic species ($As^{III}$ and $As^{V}$) constituted some 5 to 13% of the total arsenic in fauna. This converts to not more than 1 $\mu g\ g^{-1}$ inorganic arsenic for a consumable fish or crustacean, at the highest inorganic arsenic content of 0.9 $\mu g\ g^{-1}$ found in some fish and swimming crab. 'Advisable levels' for arsenic in foodstuffs suggest a 1 $\mu g\ g^{-1}$ limit on inorganic arsenic particularly where the foodstuff constitutes a regular or staple diet. Thus it could be concluded that the levels of arsenic in seafood and environment of the Pak Pa-Nang Bay may be at an acceptable level. However, until the 'total' arsenic level, set at 2 $\mu g\ As\ g^{-1}$ in Thailand is revised and full speciation analysis is available for monitoring purposes, caution is still advised.

## 5.5 Directions for future work

- **Land-Ocean Interactions**

This study has shown that arsenic from the contaminated mining area in the Pak Pa-Nang catchment is becoming widely distributed through the region, possibly as a result

of changes in the water management involving rivers and canals, particularly during the NE monsoon. It is clear from the results that the spread of arsenic throughout the complex water system of the Pak Pa-Nang needs to be more fully researched. Therefore, systematic investigations of the land-ocean transport of arsenic need to be conducted using a holistic approach in which the biogeochemistry of arsenic is linked to the hydrodynamics of the water. The aim of such an approach would be to develop calibrated models to (a) predict the dispersion of arsenic throughout the catchment under various weather conditions and (b) improve the assessment of risks to and impact on biological species and human health induced by exposure to arsenic. In order to formulate such a programme for the Pak Pa-Nang, advantage could be taken of knowledge gained during the Land-Ocean Interaction Study (LOIS) in the UK. There appear to be significant parallels between contamination transport from UK estuaries to its coastal seas and arsenic transport in the Pak Pa-Nang. This suggests that the experience gained during the LOIS programme could provide a basis for the development of a major scientific project in the Pak Pa-Nang[45]. The most important considerations for such a project are given below.

- **Land to ocean transport of arsenic in the Pak Pa-Nang region**

The key aspects of a land to ocean study in the Pak Pa-Nang are given below for each of the three separate areas of the region:-

Pak Pa-Nang River : It is essential that a comprehensive monitoring programme be established to accurately assess the fluxes of dissolved and particulate arsenic by conducting high frequency sampling campaigns in selected parts of the system, particularly during the monsoon season. The Namkhun Canal, which is identified as a major source to the wider water system, should be a prime site for investigation, although the water fluxes in the major canals also need to be monitored.

It is also important that the amount of arsenic stored in the sediments along the axis of the river, particularly near station A3, be quantified, to establish whether the arsenic in the sediments poses a long-term threat to the Bay. The assessment could be performed by taking sediment core samples of several meters depth with a vibro-corer as has been conducted in the Mersey Estuary[46]. The cores could be sectioned and analysed for arsenic species, thereby enabling the historical deposition of arsenic, say over many

decades to be detected and quantified. If long cores are obtained then there is the possibility to determine the historical deposition of other metal contaminants, such as copper, nickel and lead.

Pak Panag Bay : Seasonal changes in the dissolved arsenic concentrations in the Bay were observed, with higher concentrations being found during low flow conditions. However, it was not possible to properly assess the source of the arsenic, which is thought to be from sediment porewaters. Thus, concentrations of arsenic in the sediment porewaters of the river and estuary should be obtained and sediment-water diffusional fluxes estimated . This, together with a more accurate inventory of the arsenic load in the sediments of the Bay, would yield a better understanding of the impact of arsenic inputs from the catchment on the Bay.

The impact of arsenic, on the abundance and diversity of marine biota in the Bay, from its long-term leakage from the source needs to be fully investigated. It would be appropriate to find out if any historical data (particularly such as trends in species abundance and diversity and the amount of fish species landed) exists to estimate the long-term changes in the biological species in the estuary. Thus, it may be possible to assess the effect of arsenic pollution on trends in species abundance over several years. If no such database exists then a monitoring programme should be put in place, firstly establishing a baseline against which change can be gauged in the future.

Gulf of Thailand : Arsenic was not one of the metals chosen for investigation during the previous studies of the Gulf in 1983[32] and 1994/1995 (unpublished data). It seems clear from the results of this study and the conceptual model (Figure 5-9) that arsenic bound to fine particulate SPM passes through the Bay and into the Gulf. Also, since the waters of the Pak Pa-Nang are being re-routed via two major relief channels to the coastal waters of the Gulf that arsenic may be transported to the coastal waters through these channels. A comprehensive programme of sampling is warranted, involving sampling waters, sediments and biota in the nearshore area of the Gulf to quantify the arsenic fluxes to the Gulf. This could be coupled with an assessment of the extent to which there may be any long-term effect from arsenic on the water quality and the marine biology of the coastal area.

Model Development : The concentrations of dissolved and particulate arsenic should be combined with water fluxes in the catchment to enable arsenic fluxes to be predicted under various seasonal conditions and under various water management scenarios, such as the different water re-distribution approaches during the wet and dry seasons. As a consequence, this would involve the operation of the Pak Pa-Nang barrage, which tends to be closed at low flow and open at high flow. It would appear that it is crucial to the people of the region that a water management model be developed, refined and implemented, especially since it is unlikely that there will be an engineering solution to the mobilisation of arsenic from the source.

A primary aim of a water management model would be to restrict the further spread of arsenic in the River Basin, the Bay and the Gulf. A model would also be invaluable in assessing the impact from climate change on the mobilisation and transport of arsenic. The possibility exits that the frequency and intensity of the NE monsoons may vary in the future and a model could be used to examine the consequences of climate change, prior to the event.

- **Studies of freshwater and marine biota and food crops**

Arsenic is present in the Pak Pa-Nang basin as a range of chemical species. These include the more toxic inorganic forms (As III and V) found in high concentrations in sediments (and at lower levels in biota) together with the less to non-toxic organoarsenic types. However, only 'total' arsenic values from a limited range of sample types have been used, previously, to assess the potential impact upon human health from this environment and this did not include any speciation studies. In addition, no in-depth assessment has been made of the dietary input from arsenic species to the indigenous population of the area.

It is suggested that a full dietary survey be performed that focuses on a range of foodstuffs, together with water, that is linked to different sections of the population in terms of age and area. Speciation analysis of samples collected, will allow dietary guidelines to be devised which would be based upon the potential risk to human health from the more toxic arsenic species present to particular population-categories.

In addition, it is important to determine the actual levels of arsenic and their species in humans and other animals in our foodchain. To this end, body-fluid samples, blood and urine, should be obtained from sections of the human population (babies, children, juveniles and adults) so that 'real' body-burden mass balances are available in order to evaluate actual impacts to health. This may be extended to include fluids and tissues from other animal food sources (cows, sheep, chickens, ducks etc.) thereby allowing the potential health impacts both upon and from these sources to be estimated. Only when all this data is available can suitable foodchain models be tested and from these, recommendations be made for regulatory authorities to consider.

- **Determination of other organic arsenic compounds**

The more toxic inorganic forms of arsenic (As III and V) have been identified and measured in the sediments of the rivers and bay of the Pak Pa-Nang basin. However, the chemical nature of these solid materials is poorly understood in terms of component bioavailability/bioaccessibility. Recent work by Ruby[47; 48] and others[49; 50] has progressed the practical understanding of these terms and model digestion systems (PBET and modified PBET) which mimic stomach and intestinal processes in humans and some other mammals have been developed. It is suggested that various samples of sediments and soils from the region be evaluated in terms of their arsenic bioavailability in order to rank their potential toxic character. When extended to include various biota (flora and fauna), all these results would allow comparisons to be made with those values obtained from the dietary and body fluids (burden) study previously mentioned. Correlation models may therefore be determined, allowing future bioavailability/dietary studies of similar materials and overall complexity to be simplified.

- **Reference materials for arsenic speciation studies**

The need to determine the concentration of arsenic species rather than its total concentration has been highlighted in this study. As is the case for total arsenic determinations, the analysis of arsenic species requires suitable reference materials for the purpose of verifying the accuracy of the chosen method. There are more than 150 producers of reference material world wide[44; 51], but only one major organisation, the European Commission, BCR, has produced a speciation-related CRM[44]. The CRM 627 (fish muscle tissue) is the only product with organic arsenic compounds certified. The

production of reference materials with a range of certified arsenic species in different natural materials is urgently needed.

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**Appendix A1: Concentrations of total arsenic and arsenic species in sediments and waters and environmental parameters in the water column of the Pak Pa-Nang River Basin Sampling 2002.**

| Station | Sediment (μg As $g^{-1}$) | | | | | Dissolved As (μg $l^{-1}$) | | SPM As (μg $g^{-1}$) | DO | pH | Conductivity | Temp | Phosphate | Silicate | $NO_3$+$NO_2$ |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| | Total As | $As^{III}$ | DMA | MMA | $As^{V}$ | Total As | Inorganic | Total As | % | | μS $cm^{-1}$ | °C | μM | μM | μM |
| A1 | 13±0.7 | 0.7(6%) | nd | nd | 12(94%) | 2.4 | 2.3 | 38±1.4 | 62 | 6 | 1842 | 28.8 | 0.09 | 174 | 4.6 |
| A2 | 14±0.9 | 0.6(5%) | nd | 0.3(2%) | 14(93%) | 1.7 | 1.2 | 39.8±1.4 | 63 | 6 | 724 | 29.1 | 0.28 | 161 | 7.1 |
| A3 | 75±1.3 | 5.0(6%) | nd | 1.6(2%) | 70(92%) | 2.1 | 2.1 | 77.2±2.3 | 44 | 4.6 | 248 | 29.5 | 0.18 | 139 | 0.71 |
| A4 | 285±5.4 | 5.1(2%) | nd | nd | 277(98%) | 122 | 91 | 298±4.4 | 86 | 7 | 122 | 25.2 | 1.06 | 215 | 16.9 |
| B1 | 45±0.1 | 6.4(14%) | nd | 1.0(2%) | 39(84%) | 4.1 | 2.9 | 73±5.2 | 29 | 6.5 | 340 | 27.9 | 0.93 | 188 | 4.4 |
| B2 | 24±0.4 | 2.5(10%) | nd | 0.7(3%) | 20(87%) | 3.5 | 2.7 | 87.7±4 | 36 | 6.2 | 178 | 27.8 | 0.4 | 195 | 3.1 |
| B3 | 51±1.4 | 6.2(12%) | nd | 1.5(3%) | 44(85%) | 3.3 | 3.2 | 80.9±2.2 | 29 | 6.4 | 184 | 27.1 | 0.44 | 205 | 4.3 |
| B4 | 47±1.5 | 4.3(9%) | nd | 1.5(3%) | 41(88%) | 2.7 | 2.7 | 40.4±1.5 | 72 | 6.6 | 113 | 25.6 | 0.75 | 137 | 16 |
| B5 | 54±4.1 | 3.3(6%) | nd | 1.1(2%) | 50(92%) | 5 | 4.3 | 30.4±2 | 86 | 7 | 78 | 24.7 | 0.57 | 125 | 12.5 |
| C1 | 11±0.6 | 1.1(10%) | nd | 0.2(2%) | 10(98%) | 3.3 | 2.3 | 23±3.3 | 48 | 6.4 | 252 | 27.1 | 1.56 | 189 | 10 |
| C2 | 36±2.1 | 2.9(8%) | nd | nd | 33(92%) | 2.3 | 2.2 | 24.9±2.4 | 48 | 6.4 | 115 | 26.7 | 0.69 | 178 | 6.8 |
| C3 | 43±2.1 | 4.3(10%) | nd | 0.8(2%) | 38(88%) | 2.2 | 1.6 | 21.1±2.1 | 49 | 6.2 | 94 | 26.9 | 0.5 | 148 | 6.4 |
| D1 | 4.1±0.3 | 0.3(7%) | nd | 0.01(2%) | 3.8(91%) | 3.9 | 3.4 | 6.7±0.3 | 50 | 7.3 | 4950 | 27.5 | 3.3 | 178 | 6.8 |
| D2 | 3±0.2 | 0.1(2%) | nd | nd | 3.2(97%) | 2.7 | 2.2 | 6.1±0.3 | 56 | 6.3 | 89 | 26.7 | 4.13 | 157 | 9.8 |

nd –under limit of detection; total As = mean±standard deviation (n=3).

**Appendix A2: Concentrations of total arsenic and arsenic species in sediments and waters and environmental parameters in the water column of the Pak Pa-Nang River Basin Sampling 2003.**

| Station | Sediment (μg As $g^{-1}$) | | | | | Dissolved As (μg $l^{-1}$) | | SPM As (μg $g^{-1}$) | DO | pH | Conductivity | Temp | Phosphate | Silicate | $NO_3$+$NO_2$ | Chlorophyll |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| | Total As | $As^{III}$ | DMA | MMA | $As^{V}$ | Total As | Inorganic | Total As | % | | μS $cm^{-1}$ | °C | μM | μM | μM | μg $l^{-1}$ |
| A1 | 19±0.5 | - | - | - | - | 1.2 | 1 | 22.5±0.4 | 60 | 6.18 | 131 | 17 | 0.93 | 171 | 3.09 | 2.9 |
| A3 | 78±2.4 | - | - | - | - | 7.5 | 1 | 149±3 | 20 | 6.25 | 130 | 16 | 0.31 | 286 | 1.07 | 4.2 |
| A3A | 40±3 | - | - | - | - | 7.5 | 3.8 | 445±16 | 9 | 6.6 | 128 | 15 | 0.12 | 406 | 0.57 | 4.8 |
| A3B | 24.5±4 | - | - | - | - | 5.6 | 1.9 | 43±4 | 43 | 6.16 | 122 | 16 | 0.68 | 605 | 12.1 | 8.2 |
| A4 | 177±7 | - | - | - | - | 93 | 66 | 836±86 b/r<br>263±27 a/r | 93 | 7.36 | 192 | 11 | 1.62 | 763 | 5.44 | 2.5 |
| B2 | 39±2 | 1.8(6%) | nd | nd | 30(94%) | 1.5 | 1.4 | 84±1 | 47 | 5.77 | 112 | 17 | 1.32 | 247 | 2.18 | 2.2 |
| B4 | 50±2 | - | - | - | - | 1.6 | 1.2 | 52±1 | 57 | 7.04 | 89 | 11 | 2.6 | 220 | 6.93 | 4.5 |
| B5 | - | - | - | - | - | 1.9 | 1 | - | - | - | 152 | - | - | - | - | - |
| AB1 | - | - | - | - | - | 15 | 13 | 787±54 b/r<br>180±9 a/r | 83 | 6.34 | 61 | 11 | 0.1 | 248 | 1.4 | 1.8 |
| K1 | - | - | - | - | - | 1.4 | 0.9 | 134±3 | 56 | 6.23 | - | 14 | 0.39 | 188 | 0.9 | 6.3 |
| K2 | 60±6 | - | - | - | - | - | - | 66±3 | - | - | - | - | - | - | - | - |
| R1 | 7.3±0.8 | 2.1(26%) | nd | 0.6(7%) | 5.4(67%) | - | - | 160±5 | 5 | 6.56 | - | 14 | - | - | - | - |
| S1 | 7.6±0.7 | 0.4(6%) | nd | 0.4(6%) | 5.9(88%) | 3.8 | 2.8 | 8.4±0.4 | - | - | - | - | 1.14 | 23 | 51 | - |
| M1 | 181±4 | 4(2%) | nd | nd | 180(98%) | 148 | 138 | - | 94 | 6.36 | 43 | 12 | - | - | - | - |

nd –under limit of detection; total As = mean±standard deviation ($n$=3); b/r –sample was collected before a heavy rain; a/r –sample was collected after a heavy rain.

**Appendix A3: Concentrations of total arsenic and arsenic species in sediments and waters and environmental parameters in the water column of the Pak Pa-Nang Estuary Sampling 2001.**

| Station | Sediment (μg As $g^{-1}$) | | | | | Dissolved As (μg $l^{-1}$) | | pH | Temp | Salinity | Conductivity | Phosphate | Silicate | $NO_3$+$NO_2$ |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| | Total As | $As^{III}$ | DMA | MMA | $As^{V}$ | Total | Inorganic | | °C | psu | μS $cm^{-1}$ | μM | μM | μM |
| ppn 1 | 14.1±2.5 | 11 | nd | nd | 89 | 4.58 | 4.58 | 7.99 | 29.6 | 24.5 | 41295 | 0.14 | 8.88 | 17.96 |
| ppn 2 | 11.7±2.1 | 10 | nd | nd | 90 | 12.08 | 7.08 | 7.6 | 31.4 | 23.7 | 39946 | 1.13 | 50.42 | 1.52 |
| ppn 3 | 12.9±2.6 | 11 | nd | nd | 89 | 11.25 | 6.25 | 8.17 | 30 | 26.2 | 44160 | 0.52 | 20.19 | 1.25 |
| ppn 4 | 14±3.2 | 10 | nd | nd | 90 | 3.75 | 5.42 | 7.82 | 30.1 | 27.9 | 47025 | 0.94 | 32.57 | 3.79 |
| ppn 5 | 4.1±1 | 12 | nd | nd | 88 | 4.58 | 3.75 | 8.12 | 30 | 29.9 | 50396 | 0.61 | 15.84 | 2.65 |
| ppn 6 | 6.8±2.3 | 12 | nd | nd | 88 | 8.75 | 5.42 | 7.98 | 29.5 | 25.8 | 43486 | 0.73 | 17.8 | 3.33 |
| ppn 7 | 12±2.3 | 20 | nd | nd | 80 | 6.25 | 5.42 | 8.4 | 31.2 | 23.6 | 39778 | 3.26 | 9.72 | 1.02 |
| ppn 8 | 8.8±1.6 | 14 | nd | nd | 86 | 12.08 | 7.92 | 8.21 | 30.7 | 28.6 | 48205 | 0.21 | 20.47 | 0.91 |
| ppn 9 | 7.9±1.4 | 12 | nd | nd | 88 | 4.58 | 2.92 | 8.07 | 29.3 | 31.4 | 52925 | 0.14 | 14.95 | 0.7 |

nd –under limit of detection; total As = mean±standard deviation (n=3); psu = practical salinity unit.

**Appendix A4: Concentrations of total arsenic and arsenic species in sediments and waters and environmental parameters in the water column of the Pak Pa-Nang Estuary Sampling 2002.**

| Station | Sediment (μg As $g^{-1}$) | | | | | Dissolved As (μg $l^{-1}$) | | SPM As (μg $g^{-1}$) | DO | pH | Temp | Salinity | Conductivity | Phosphate | Silicate | $NO_3$+$NO_2$ | Chlorophyll |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| | Total As | $As^{III}$ | DMA | MMA | $As^{V}$ | Total | Inorganic | Total | % | | °C | psu | μS $cm^{-1}$ | μM | μM | μM | μg $l^{-1}$ |
| ppn 1 | 11.1±0.3 | 1.2(8%) | nd | nd | 10(92%) | 2.1 | 1.3 | 6.4±0.4 | 64 | 7.1 | 28.7 | 4.5 | 8130 | 0.2 | 126 | 1.9 | - |
| ppn 2 | 10.6±0.6 | 1.3(8%) | nd | nd | 11(92%) | 2.4 | 0.7 | 5.8±0.5 | 100 | 7.8 | 27.8 | 6.3 | 11070 | 0.2 | 116 | 4.9 | - |
| ppn 3 | 16.6±0.3 | 1.1(6%) | nd | nd | 16(94%) | 3 | 2.1 | 6±0.6 | 66 | 7.1 | 26.9 | 0.4 | 810 | 1.34 | 152 | 8.7 | - |
| ppn 4 | 19.7±0.3 | 1.3(6%) | nd | nd | 20(94%) | 3.7 | 2.9 | 3.8±0.4 | 90 | 7.3 | 27.4 | 5.5 | 9880 | 1.54 | 130 | 13 | 1.2 |
| ppn 5 | 20.3±1 | 1.5(7%) | nd | nd | 20(93%) | 2.7 | 2.1 | 6.8±0.8 | 103 | 8 | 28.5 | 16.6 | 27020 | 0.25 | 91.8 | 6.9 | 2.3 |
| ppn 6 | 19.4±0.3 | 1.5(8%) | nd | nd | 18(92%) | 1.1 | 0.9 | 6±0.4 | 88 | 7.4 | 28.6 | 9.5 | 16220 | 0.15 | 115 | 3 | 0.8 |
| ppn 7 | 19.8±0.6 | 1.5(8%) | nd | nd | 19(92%) | 2.1 | 2.1 | 4.2±0.1 | 99 | 7.6 | 28.7 | 7.8 | 13500 | 0.15 | 146 | 3.1 | 1.7 |
| ppn 8 | 20±0.5 | 1.4(7%) | nd | nd | 17(93%) | 2.4 | 1.9 | 4.4±0.7 | 95 | 7.8 | 28.8 | 10.3 | 17600 | 0.3 | 107 | 9.2 | 1.6 |
| ppn 9 | 8.4±0.4 | 0.6(8%) | nd | nd | 7.2(92%) | - | - | 5.4±0.2 | - | - | - | - | - | - | - | - | - |

nd –under limit of detection; total As = mean±standard deviation (n=3); psu = practical salinity unit.

**Appendix A5: Concentrations of total arsenic and arsenic species in SPM and waters and environmental parameters in the water column of the Pak Pa-Nang Estuary Sampling 2003.**

| Station | Dissolved As ($\mu g\ l^{-1}$) | | SPM As ($\mu g\ g^{-1}$) | DO | pH | Temp | Salinity | Conductivity | Phosphate | Silicate | $NO_3$+$NO_2$ | Chlorophyll |
|---|---|---|---|---|---|---|---|---|---|---|---|---|
| | Total | Inorganic | Total | % | | °C | psu | $\mu S\ cm^{-1}$ | µM | µM | µM | $\mu g\ l^{-1}$ |
| 1 | 1.64 | 1.34 | 11±0.4 | 73 | 7.77 | 27.8 | 12.6 | 21237 | 1.1 | 68 | 4.2 | 15 |
| 12 | 1.21 | 1.15 | 2.8±0.2 | 97 | 7.84 | 28.1 | 22.4 | 37755 | 0.4 | 30 | 1.5 | 2.2 |
| 27 | 1.2 | 1.2 | 8.4±0.2 | - | - | - | - | - | 0.9 | 57 | 4.1 | 11 |
| 31 | 0.98 | 0.86 | 17±0.6 | - | - | - | - | - | 0.2 | 13 | 0.6 | 5.5 |
| 34 | 1.07 | 0.86 | 12±0.3 | 94 | 7.73 | 28.2 | 25 | 42138 | 0.2 | 10 | 0.4 | 6.1 |
| 48 | 1.73 | 0.81 | 21±0.8 | - | - | - | - | - | 2.1 | 81 | 5.9 | 2.1 |
| 55 | 1.26 | 0.86 | 16±0.6 | - | - | - | - | - | 0.7 | 32 | 2.1 | 5 |
| 59 | 0.28 | 0.24 | 9.2±0.2 | 96 | 8.1 | 28 | 25 | 42137 | 0.2 | 17 | 0.9 | 2.5 |
| 122 | 1.12 | 0.96 | 44±2 | - | - | - | - | - | 0.7 | 64 | 1.6 | 8.9 |

total As = mean±standard deviation (n=3); psu = practical salinity unit.

**Appendix A6: Vertical distributions of total arsenic in sediments of the Pak Pa-Nang River Basin and its estuary; units are in μg $g^{-1}$ dry weight, mean ± standard deviation (n=3).**

| | Riverine sediments | | | | | | | Estuarine sediments | | | | |
|---|---|---|---|---|---|---|---|---|---|---|---|---|
| Depth (cm) | A1 | A3 | A3A | B3 | B4 | C1 | C3 | PPN1 | PPN2 | PPN3 | PPN4 | CORE 35 |
| 0-2 | 15.1±0.7 | 78±5 | 34±4 | 49±1.4 | 54±1.5 | 8±0.6 | 43±2.1 | 13.2±2 | 9.2±0.2 | 12.7±2.6 | 13.8±1.5 | 8.7±0.4 |
| 2-4 | 13.9±0.4 | 59±3 | 26±1 | 46±2.1 | 48±3.4 | 10.6±0.4 | 40±1.3 | 14.1±2.2 | 8.8±0.5 | 12.6±2.6 | 14.2±1.5 | 8.7±0.6 |
| 4-6 | 12.5±0.5 | 67±6 | 23.3±2 | 45±3.4 | 46±2.3 | 8.2±0.4 | 51±1.8 | 12.4±1.8 | 10±0.6 | 12.5±3 | 14.3±1.6 | 8.8±0.3 |
| 6-8 | 10.5±0.2 | 56±3 | 16.7±1 | 48±4.1 | 49±3.4 | 7.8±0.2 | 41±1.9 | 14.1±2.3 | 17±0.6 | 12.3±3.1 | 14.2±1.7 | 8.3±0.8 |
| 8-10 | 14.3±0.3 | 67±2 | 11.5±2 | | | 8±0.6 | | 13.6±2.2 | 16±0.5 | 8.6±2.8 | 13.9±1.7 | 10.5±0.5 |
| 10-12 | 12.3±0.2 | 56±3 | 15±1 | | | 9.8±0.2 | | 11±1.9 | 18±0.9 | 7.3±2.6 | 14.2±1.6 | 11.4±0.4 |
| 12-14 | 12.8±0.5 | 69±2 | 12.2±0.5 | | | 9.8±0.3 | | 12±2.2 | 19±0.7 | 6.3±2.5 | 15.5±1.9 | 9.4±0.3 |
| 14-16 | 13±0.4 | 52±1 | 12±1 | | | 7±0.2 | | 10.9±1.8 | 32±2 | 7.5±2.7 | 15±1.8 | 15±0.8 |
| 16-18 | 12.8±0.3 | | | | | 8±0.4 | | 10.8±1.8 | 30±0.5 | 9±2.9 | 15.7±2.1 | 9.5±0.4 |
| 18-20 | 12.6±0.3 | | | | | 8.6±0.2 | | 11.5±2.1 | 23±3 | 8±2.9 | 16.1±2.3 | |
| 20-22 | | | | | | | | | | | 14.6±2.1 | |
| 22-24 | | | | | | | | | | | 14.2±2.2 | |

**Appendix B1: Habitat and feeding characteristic of fish and shellfish samples collected from the Pak Pa-Nang Estuary.**

| Name | Habitat | Feeding characteristic | Ref. |
|---|---|---|---|
| Catfish *Plotosus canius* | Found mostly in estuaries and lagoons, and sometimes up rivers in nearly fresh waters | Feeds on crustaceans, mollusks and fishes | 1 |
| Mullet *Mugil cephalus* | They are coastal species that often enters estuaries and rivers, usually in schools over sand or mud bottom | Mainly diurnal, feed on zooplankton, benthic organisms and detritus | 2 |
| Croaker *Johnius belangerii* | Inhabits coastal waters and estuaries | Feeds on invertebrates, particularly benthic worms | 3 |
| Benthic fish | Benthic coastal waters and estuaries | - | 4 |
| Sardines *Escualosa thoracata* | Forms schools in coastal waters and strongly migratory | Feeds mainly on phytoplankton (especially diatoms) and small crustaceans | 5 |
| Leopard Scat *Scatophagus argus* | Natural embayments, brackish estuaries and the lower reaches of freshwater streams, frequently occurring among mangroves | Feed on worms, crustaceans, insects and plant matter | 6 |
| Mud crab *Scylla serrata* | Live in tidal flats and rivers lined with mangroves, They favour a soft muddy bottom | Mud Crabs are omnivorous scavengers and are also cannibalistic, eating other crabs as well as barnacles, bivalves and dead fish. | 7 |
| Swimming crab *Portunus pelagicus* | Flower crabs are common in the seagrass lagoon, they bury themselves under sand or mud | These crabs hunt fish and other swimming animals at high tide | 8 |
| Tiger prawn *Penaeus monodon* | They are common at 10-20 metres of water over coarse sediments | Tiger prawns feed primarily at night. Their diet consists of molluscs, crustaceans and polychaete worms | 9 |
| Freshwater prawn *Macrobrachium rosenbergii* | Live in the benthic of the rivers and adjoining brackish waters | The larvae feed on zooplankton, but the juveniles and adults are omnivorous. They ingest a variety of food items of both plant and animal origin as well as detritus. The common items of food include aquatic worms, small molluscs and crustacea | 10 |

Mullet
*Mugil cephalus*

Catfish
*Plotosus canius*

Benthic fish

Sardines
*Escualosa thoracata*

Croaker
*Johnius belangerii*

Leopard Scat
*Scatophagus argus*

Mud crab
*Scylla serrata*

Swimming crab
*Portunus pelagicus*

Tiger prawn
*Penaeus monodon*

Freshwater prawn
*Macrobrachium rosenbergii*

**<u>Appendix B2</u>: Pictures, common name and scientific name of fish and shellfish samples collected from the Pak Pa-Nang Estuary.**

## References for Appendix B


1 Agbayani, E. '*Plotosus canius* Gray eel-catfish' [Online] http://www.fishbase.org/Summary/SpeciesSummary.cfm?&genusname=Plotosus+&speciesname=canius [28th February 2004].

2 Agbayani, E. '*Mugil cephalus* Flathead mullet' [Online] http://www.fishbase.org/Summary/SpeciesSummary.cfm?ID=785&genusname=Mugil&speciesname=cephalus [29th February 2004].

3 Agbayani, E. '*Johnius belangerii* Belanger's croaker' [Online] http://www.fishbase.org/Summary/SpeciesSummary.cfm?&genusname=Johnius+&speciesname=belangerii [28th February 2004].

4 Agbayani, E. 'List of common names with blenny' [online] http://www.fishbase.org/ComNames/CommonNameSearchList.cfm [14th June 2004].

5 Agbayani, E. '*Sardinella longiceps* Indian oil sardine' [Online] http://www.fishbase.org/Summary/SpeciesSummary.cfm?ID=1511&genusname=Sardinella&speciesname=longiceps [28th February 2004].

6 Agbayani, E. '*Scatophagus argus* Spotted scat' [Online] http://www.fishbase.org/Summary/SpeciesSummary.cfm?genusname=Scatophagus&speciesname=argus [29th February 2004].

7 Department of Primary Industries Australia 'Mud crab (mangrove crab) *Scylla serrata*' [Online] http://www.dpi.qld.gov.au/fishweb/2454.html [29th February 2004].

8 Tan, Leo W. H. and Ng, Peter K. L 'Flower crabs *Portunus pelagicus* Family Portunidae' [Online] http://mangrove.nus.edu.sg/pub/seashore/text/202.htm [29th February 2004].

9 'Tiger Prawn' [Online] http://www.sea-ex.com/fishphotos/prawn%2C3.htm [14th June 2004].

10 '*Macrobrachium rosenbergii*' [Online] http://www.freshwaterprawn.com/main.asp [29th February 2004].

## Appendix C: Courses, conferences attended and presentations.

- **Courses attended and passed at the University of Plymouth**

Research Methods: Module EAR 5101 (10 Credits)
Research Skills: Module IMS 5101 (10 Credits)
Ecological Toxicology: Module BIO 5108 (10 Credits)
Principles & Applications in Electron Microscopy: Module BIO 5102 (10 Credits)

- **Workshops attended**

Coupled Techniques for Elemental Speciation: Short Course in 11th Biennial National Atomic Spectroscopy Symposium (BNASS 2002), held at the Loughborough University, UK, July 2002.

Measurement of Bioaccessibility of Metals in Soils: Short Course in 12th Biennial National Atomic Spectroscopy Symposium (BNASS 2004), held at the University of Plymouth, UK, July 2004.

- **Conferences attended and presentations**

Attended the 3rd IGBP-LOICZ International Symposium on 'Functioning of Coastal Ecosystems in Various Geographical Regions', held in Gdynia, Poland, June 2001.
Poster presentation entitled '*Distribution and speciation of arsenic in marine organisms influenced by mining activities*'.

Attended the Analytical Research Forum, held at the University of East Anglia, UK, July 2001.
Poster presentation entitled '*Distribution and speciation of arsenic in the Pak Pa-Nang Estuary*'.

Attended the 40th Annual Meeting of British Sedimentary Research Group (BSRG 2001), held at the University of Plymouth, UK, December 2001.
Poster presentation entitled '*Metal distributions in the sediments of the Pak Pa-Nang Estuary, Thailand*'.

Attended the Seminar of Atomic Spectroscopy Group, Department of Environmental Sciences, University of Plymouth, UK, 2001 and 2002.
Oral presentation entitled '*Speciation of arsenic in fish samples by HPLC-ICP-MS'* at the '*Weekly Departmental Seminar*'.

Attended the 11th Biennial National Atomic Spectroscopy Symposium (BNASS 2002), held at Loughborough University, UK, July 2002.
Poster presentation entitled '*Distribution and speciation of arsenic in biota samples from the Pak Pa-Nang Estuary, Thailand*', Awarded "best student presentation prize" in the 'Environmental Posters' section.

Attended the Analytical Research Forum, held at the Kingston University, UK, July 2002.
Poster presentation entitled '*Speciation of arsenic in marine fish of the Pak Pa-Nang Estuary, Thailand*',

Attended the Challenger Centenary Conference: Marine Science 2002, held at the University of Plymouth, UK, September 2002.
Poster presentation entitled '*Speciation of arsenic in samples from the Pak Pa-Nang Estuary, Thailand and implications for human health*', Extension to the poster: www.env.plym.ac.uk/research/2002/challenger_2002_sr.htm

Attended the 5th Progress in Chemical Oceanography meeting, held at University of East Anglia, UK, July 2003.
Oral presentation entitled '*Determination of arsenic speciation in fish, crustacean and sediment samples from Thailand using HPLC coupled with ICP-MS'*.

Attended the 5th International Symposium on Speciation of Elements in Biological, Environmental and Toxicological Sciences, held at Granada, Spain, September 2003.
Oral presentation entitled '*Determination of arsenic speciation in fish, crustacean and sediment samples from Thailand using HPLC coupled with ICP-MS'*.

Work presentation in the International Symposium on Coastal Oceanography, held in Japan, December 2003.
Oral presentation by a college entitled '*Speciation of arsenic in Pak Pa-Nang Bay, southern Thailand.*

Attended the 12th Biennial National Atomic Spectroscopy Symposium (BNASS 2004), held at the University of Plymouth, UK, July 2004.

Poster presentation entitled "*Determination of Arsenic Speciation in Fish, Plant and Sediment Samples from Thailand Using HPLC Coupled with ICP-MS*".

## Appendix D: Publish works.

Rattanachongkiat, S., Millward, G.E. and Foulkes, M.E. (2004) Determination of arsenic species in fish, crustacean and sediment samples from Thailand using high performance liquid chromatography (HPLC) coupled with inductively coupled plasma mass spectrometry (ICP-MS) *Journal of Enviromental Monioring*, **6**, 254-261.

Utoomprurkporn, W., Millward, G.E., Foulkes, M.E., Rattanachongkiat, S., Taiyaqupt, M. and Tantichodok, P. (2003) Seasonality in arsenic biogeochemistry: Pak Panang Bay, southern Thailand. Paper presented in Goldschmidt Conference 2003, Japan. (Abstract reported in *Geochimica Cosmochimita Acta*, **67**, A507)

PAPER


# Determination of arsenic species in fish, crustacean and sediment samples from Thailand using high performance liquid chromatography (HPLC) coupled with inductively coupled plasma mass spectrometry (ICP-MS)†

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Suitable techniques have been developed for the extraction of arsenic species in a variety of biological and environmental samples from the Pak Pa-Nang Estuary and catchment, located in Southern Thailand, and for their determination using HPLC directly coupled with ICP-MS. The estuary catchment comprises a tin mining area and inhabitants of the region can suffer from various stages of arsenic poisoning. The important arsenic species, AsB, DMA, MMA, and inorganic arsenic (As III and V) have been determined in fish and crustacean samples to provide toxicological information on those fauna which contribute to the local diet. A Hamilton PRP-X100 anion-exchange HPLC system employing a step elution has been used successfully to achieve separation of the arsenic species. A nitric acid microwave digestion procedure, followed by carrier gas nitrogen addition- ($N_2$)-ICP-MS analysis was used to measure total arsenic in sample digests and extracts. The arsenic speciation of the biological samples was preserved using a Trypsin enzymatic extraction procedure. Extraction efficiencies were high, with values of 82–102% (As) for fish and crustacean samples. Validation for these procedures was carried out using certified reference materials. Fish and crustacean samples from the Pak Pa-Nang Estuary showed a range for total arsenic concentration, up to 17 μg $g^{-1}$ dry mass. The major species of arsenic in all fauna samples taken was AsB, together with smaller quantities of DMA and, more importantly, inorganic As. For sediment samples, arsenic species were determined following phosphoric acid (1 M $H_3PO_4$) extraction in an open focused microwave system. A phosphate-based eluant, pH 6–7.5, with anion exchange HPLC coupled with ICP-MS was used for separation and detection of $As^{III}$, $As^{V}$, MMA and DMA. The optimum conditions, identified using an estuarine sediment reference material (LGC), were achieved using 45 W power and a 20 minute heating period for extraction of 0.5 g sediment. The stability and recovery of arsenic species under the extraction conditions were also determined by a spiking procedure which included the estuarine sediment reference material. The results show good stability for all species after extraction with a variability of less than 10%. Total concentrations of arsenic in the sediments from the Pak Pa-Nang river catchment and the estuary covered the ranges 7–269 μg $g^{-1}$and 4–20 μg $g^{-1}$ (dry weight), respectively. $As^{V}$ was the major species found in all the sediment samples with smaller quantities of $As^{III}$. The presence of the more toxic inorganic forms of arsenic in both sediments and biota samples has implications for human health, particularly as they are readily 'available'.

## 1. Introduction

Many recent studies of arsenic have focused on speciation because its toxicity differs greatly among its species.[1] Inorganic species, arsenite ($As^{III}$) and arsenate ($As^{V}$) are extremely toxic and have also been classified as being carcinogenic.[2,3] Organic arsenic species *e.g.*, monomethylarsonic acid (MMA) and dimethylarsinic acid (DMA) have been classified as being cancer promoters.[4] Arsenobetaine (AsB), the major species found in organisms, is relatively non-toxic and can be excreted un-metabolised from the human body.[5–7]

Drinking water and dietary intake are the two major sources of arsenic ingestion in humans. Seafood is a major source of arsenic exposure from the diet, which makes the research of arsenic speciation in seafood an important study area.[8] The importance of speciation is further emphasised when it is known that the WHO recommendation states that the daily oral intake of "inorganic" arsenic should not exceed 2 μg $kg^{-1}$ body weight.

The Pak Pa-Nang Estuary is located in southern Thailand and its catchment comprises a former tin mining area, with concomitant arsenic-bearing minerals. Drainage from the high arsenic concentration deposits and spoil tips of the mined area could affect water and sediment quality in the rivers and the bay, which is biologically productive, including rice paddy fields, substantial fish stocks, mussel and crustacea aquaculture.[9]

Coupling of High-Performance Liquid Chromatography (HPLC) with Inductively Coupled Plasma Mass Spectrometry (ICP-MS) has received interest because it combines the efficient separation and detection of arsenic species.[10–17] The advantages of ICP-MS are its high level of sensitivity and its time resolved mode that allows on-line real time analysis of the HPLC eluants.

Enzymatic extraction using trypsin provides a high extraction efficiency whilst maintaining the integrity of the arsenic species in the proteinaceous sample. Previously, this extraction technique has been used successfully to extract arsenic species from fish samples[14] and baby food samples.[17] The stability of the arsenic species using the extraction procedure is important for speciation studies.

Phosphoric acid has been used successfully for the extraction of arsenic species from soils and sediments with high efficiency and without modification.[15,18]

† Presented at the 5th International Symposium on Speciation of Elements in Biological, Environmental and Toxicological Sciences, Almuñécar (Granada), Spain, 13–16 September 2003.

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**Table 1** Acid digestion procedure for total available arsenic

| Step | Procedure |
|---|---|
| 1 | Weigh 0.25 g freeze dried sample directly into a digestion bomb (or transfer the residual solid left from the enzymatic extraction [Table 3] to a digestion bomb) |
| 2[a] | Add conc. (69%) Nitric acid (4 ml) and hydrogen peroxide 37% (1 ml) then cap the bomb loosely and leave overnight for pre-digestion |
| 3[b] | After pre-digestion, cap the bomb securely and heat in a microwave oven at medium power (*ca.* 700 W) for 3 minutes (for 4 bombs). Following a short cool-down period and release of pressure, the bombs are heated for another 2 minutes to complete the digestion |
| 4 | After cooling transfer the digest to a 50 ml volumetric flask, spike with the internal standard and dilute to volume using 2% nitric acid |
| 5 | The digest is ready for analysis |

[a] For some materials, heating at this early stage may lead to an explosion, hence the need for a cool pre-digestion stage. [b] A completed digest (concentrated) of a biological material is usually dark green. If the digest is still yellow, it may be necessary to heat for a longer period. Occasionally it may be necessary to add a further aliquot (1 ml) of nitric acid in addition to the second heating step to ensure complete digestion.

This paper reports the application of HPLC separation and ICP-MS detection and also of enzymatic and phosphoric acid extractions to determine arsenic species in both biological and sediment reference materials. The optimised techniques were then applied to various fish, crustacea and sediments from the Pak Pa-Nang Estuary and its catchment areas to assess the impact from mining activities.

## 2. Experimental

### 2.1 Standards, reagents and materials

DORM 1 (dogfish muscle) and TORT 1 (lobster hepatopancreas) were obtained from the National Research Council, Ottawa, Canada. LGC 6137 estuarine sediment reference material was obtained from the Laboratory of the Government Chemist, Middlesex, United Kingdom.

All solutions were prepared with Milli-Q (18 MΩ cm) water. $As^V$ standards were prepared from a high purity stock solution of 996 ± 2 mg $dm^{-3}$ (Aldrich, Gillingham, Dorset, UK). Indium stock standard was obtained from BDH laboratory supplies, Poole, UK. DMA standard solutions were prepared from Cacodylic acid, sodium salt 98% purchased from Sigma, Gillingham, Dorset, UK. The MMA standard was kindly donated by Antonio Moreda-Pineiro, University of Santiago de Compostela, Spain. For arsenic speciation analysis in fish, AsB, DMA, MMA, and inorganic As standards were prepared in 0.1 M $NH_4HCO_3$ (pH 8). For arsenic speciation analysis in sediment $As^V$, $As^{III}$, DMA and MMA standards were prepared in 0.25 M $H_3PO_4$. The $As^V$ and $As^{III}$ standards were prepared freshly each time for analysis in order to avoid oxidation of $As^{III}$ to $As^V$.

Trypsin (from bovine pancreas) powder was purchased from Sigma (Sigma, Gillingham, Dorset, UK). Hydrogen peroxide 37% was purchased from Merck, Poole, Dorset, UK. Nitric acid (Aristar), ammonium hydrogen carbonate (AnalaR) and sodium hydroxide pellets (AnalaR) were also purchased from Merck, Poole, Dorset, UK.

An analytical column and a guard column were packed with Hamilton PRP-X100 resin purchased from Phenomenex, Cheshire, UK. Columns were packed using a wet packing technique in 5 mM $Na_2SO_4$ pH 10 at 2000–3000 pounds per square inch pressure.

Glassware and plastic centrifuge tubes were pre-cleaned by leaving for two days in 5% Decon 90 (Merck) in Milli Q water and 10% v/v nitric acid in Milli Q water then rinsed with Milli Q water prior to use.

### 2.2 Sample preparation

**2.2.1 Sampling and sample pretreatment.** Fish and crustacean samples were collected using local fishing boats from the Pak Pa-Nang Estuary, Thailand in August 2001 and December 2002. The samples (whole body for sardine and swimming crab) were cleaned, catfish muscle tissue was separated from bone and tiger prawn muscle tissue was separated from shell. Sediment samples were collected using a grab sampler and also a core sampler which could retrieve settled sediment down to 50 cm depth; the latter to give a temporal profile. It is noted that all samples were stored in ice on board boat and freeze-dried/processed within hours of collection. All the samples were frozen at −40 °C for 12 h in a freezer then placed in a freeze drier for 48 h. The dried fish, crustacean and sediment samples were ground using an agate pestle and mortar to a fine powder. The samples were then stored in brown bottles and placed in a desiccator in order to avoid exposure to light and moisture until required for analysis.

**2.2.2 Acid digestion for total arsenic.** Total available arsenic in the fish, sediment samples and residual solids from enzymatic extractions were digested by a microwave-assisted digestion in a Teflon bomb using nitric acid and hydrogen peroxide. The procedure is shown in Table 1. The digested solutions were spiked with a solution of indium, internal standard to a concentration of 100 μg $l^{-1}$ prior to their determination by ICP-MS.

**2.2.3 Phosphoric acid extraction for arsenic speciation in sediments.** The extraction process follows from previous studies of the arsenic speciation in soils and sediments.[15,18] A microwave digester system, Synthewave 402 (Prolabo, Fontanay-Sous-Bois Cedex, France) was used for the extraction. This is a focused microwave system in an open quartz flask with adjustable power and heating time. To stir the sediment during the extraction, a glass-stirring paddle was used accompanied by flask rotation. The LGC 6137 Estuarine sediment certified reference material was used to optimize the procedure. Samples (0.5 g) were extracted using the optimized process shown in Table 2.

**Table 2** Phosphoric acid extraction procedure to determine As species in sediment samples

| Step | Procedure |
|---|---|
| 1 | Weigh 0.5 g freeze dried sediment sample directly into a beaker |
| 2 | Add 1 M $H_3PO_4$ (25 ml) and stir with a glass rod then transfer to the extraction vessel |
| 3 | Place the vessel into the microwave digester and heat at 45 W for 20 minutes |
| 4 | Let the sample cool down then transfer to a centrifuge tube |
| 5 | Centrifuge the sample at 2500 rpm for 10 min |
| 6 | Pour the extract into a volumetric flask (100 ml), spike with internal standard solution and dilute to volume using Milli-Q water |
| 7 | The extract is ready for analysis |

**Table 3** Enzymatic extraction procedure for fish and crustacean samples

| Step | Procedure |
|---|---|
| 1 | Weigh 0.25 g freeze dried sample and 0.1 g trypsin directly into a Potter homogenizer |
| 2 | Add 0.1 M $NH_4HCO_3$ (10 ml) and homogenise with the sample; then transfer to a plastic centrifuge tube |
| 3 | Add another 10 ml of 0.1 M $NH_4HCO_3$ to rinse the homogenising tube then transfer to the same centrifuge tube |
| 4 | Cap the tube and leave in a shaking water bath at 37 °C for 12 h |
| 5 | Centrifuge the sample at 2000 rpm for 20 min |
| 6[a] | Pour the extract into a volumetric flask (25 ml) spike with caesium internal standard solution and dilute to volume with 0.1 M $NH_4HCO_3$ |
| 7 | The extract is ready for analysis (the extract can be kept in darkness at 4 °C for no longer than 1 week) |

[a] Keep the residual solid for residue-arsenic analysis.

**2.2.4 Enzymatic extraction for arsenic speciation in fish and crustacea.** Trypsin is an enzyme that is well known as a protein degrader. It breaks down dietary protein molecules to their component peptides and amino acids. Trypsin has been found to work well in a slightly alkaline environment, about pH 8 at 37 °C (human body temperature). Arsenic species in the samples were extracted using the trypsin in 0.1 M ammonium hydrogen carbonate ($NH_4HCO_3$), following the method previously developed by Branch *et al.*[14] The procedure is shown in Table 3. On this occasion, caesium was used as an internal standard, because under these pH conditions, indium is unstable.

### 2.3 Analytical methods

For total arsenic a VG PlasmaQuad 2 (VG Elemental, Winsford, Cheshire, UK) was used. The combination of chlorine introduced *via* the sample with argon from the plasma can give rise to the formation of $^{40}Ar^{35}Cl^+$, which interferes with the monoisotopic $^{75}As^+$. The problem was overcome by adding the molecular gas, nitrogen, to the nebuliser gas (approximately 4.5% v/v) according to the method used successfully by Hill *et al.*[19] A gas blender (Signal series 850, Signal, Camberley, Surrey, UK) was used for the nitrogen gas addition. Indium standard was used as an internal standard at a final concentration of 100 ng $ml^{-1}$. The mass spectrometer was set to measure ion intensities (peak jumping) at the analysed mass *m/z* 75 ($^{75}As^+$). The operating conditions are given in Table 4. Additionally, the signal intensity is sampled at *m/z* 115 ($^{115}In^+$), used for internal standardisation. The liquid sample is introduced by a peristaltic pump at a flow rate of 1.5 ml $min^{-1}$.

For the chromatographic separation of enzymatic extracts and phosphoric acid extracts, a Waters 6000A chromatographic pump (Waters, Milford, MA, USA) was used with a 250 × 4.6 mm stainless steel column packed with 10 μm particle size Hamilton PRP-X100 anion-exchange resin. The column was protected by a 50 × 4.6 mm guard column packed with the same material. The specification of the system and operating conditions are given in Table 4. The ICP-MS was set to acquire time-resolved data. Data for arsenic (*m/z* 75) were recorded using the peak jumping acquisition and displayed as mass-intensity-time plots. The concentrations of each species were calculated using peak areas and were compared with standard solutions that were injected alternately. In addition, the spiking of sample extracts with known standards was also employed to take account of those matrix effects upon retention time and response factors.

## 3. Results and discussion

### 3.1 HPLC optimization for the enzymatic extracts of fish and crustacean samples

Fig. 1a shows the chromatogram of four standard arsenic species, AsB, DMA, MMA and inorganic As, respectively. This separation was achieved successfully using an anion-exchange stationary phase column. After injection, an arsenic compound is eluted from the column using the low concentration $Na_2SO_4$ solution as mobile phase. The pH of the mobile phase was selected according to the $pK_a$ values of the arsenic compounds. The $pK_a$ value of 9.2 for $As^{III}$ indicated that this species is ionized at pH 10 in the mobile phase used in this work.[16] The analysis is complete in 900 seconds using two concentrations of a competitive anion-containing mobile phase, $Na_2SO_4$, **(a)**: 5mM and **(b)**: 50mM (Table 4). AsB and DMA are eluted with concentration **(a)** then MMA and Inorganic As are eluted when the mobile phase is switched to concentration **(b)**. The elution of all four species is possible using one concentration of mobile phase but, if concentration **(a)** is selected MMA and inorganic As take an unacceptably long time to elute. Conversely if concentration **(b)** is selected for isocratic elution, all the four species will be eluted together in 300 seconds without

**Table 4** ICP-MS and HPLC operating conditions

| | |
|---|---|
| **ICP-MS** | |
| Forward power/W | 1350 |
| Gas flows/l $min^{-1}$ | |
| Nebuliser | 1.0 |
| Auxiliary | 1.25 |
| Coolant | 13 |
| Nebuliser type | Ebdon, high solids |
| $N_2$ addition | 4–4.5% v/v of nebuliser gas flow |
| Isotope masses | $^{75}As$, $^{115}In$ and $^{133}Cs$ |
| **HPLC conditions for enzyme extracts** | |
| Column | Hamilton resin PRP-X100 10 μm (250 × 4.6 mm) |
| Guard column | Hamilton resin PRP-X100 10 μm (50 × 4.6 mm) |
| Standard solutions | 200 μg $l^{-1}$ AsB, DMA, MMA and inorganic arsenic |
| Injection loop/μl | 100 |
| Flow rate/ml $min^{-1}$ | 1.5 |
| Mobile phases | (a) 5 m mol $l^{-1}$ $Na_2SO_4$ pH 10–10.5[a]<br>(b) 50 m mol $l^{-1}$ $Na_2SO_4$ pH 10–10.5[a] |
| Mobile phase program | |
| Isocratic elution | Mobile phase (a) for 360 seconds |
| Step gradient | Mobile phase (b) for 180 seconds |
| Re-equilibrate | Mobile phase (a) until finish |
| Total time required for chromatogram | 900 seconds |
| **HPLC conditions for phosphoric acid extracts** | |
| Column | Hamilton resin PRP-X100 10 μm (250 × 4.6 mm) |
| Guard column | Hamilton resin PRP-X100 10 μm (50 × 4.6 mm) |
| Standard solutions | 100 μg $l^{-1}$ $As^{III}$, DMA, MMA and $As^V$ |
| Injection loop/μl | 20 |
| Flow rate/ml $min^{-1}$ | 1.5 |
| Mobile phases | (a) 2 m mol $l^{-1}$ $H_3PO_4$ pH 7.5[a]<br>(b) 50 m mol $l^{-1}$ $H_3PO_4$ pH 6[a] |
| Mobile phase cycle | |
| Isocratic elution | Mobile phase (a) for 360 seconds |
| Step gradient | Mobile phase (b) for 180 seconds |
| Re-equilibrate | Mobile phase (a) until finish |
| Total time required for chromatogram | 900 seconds |

[a] Adjusted with concentrated ammonia (0.88 s.g) solution.

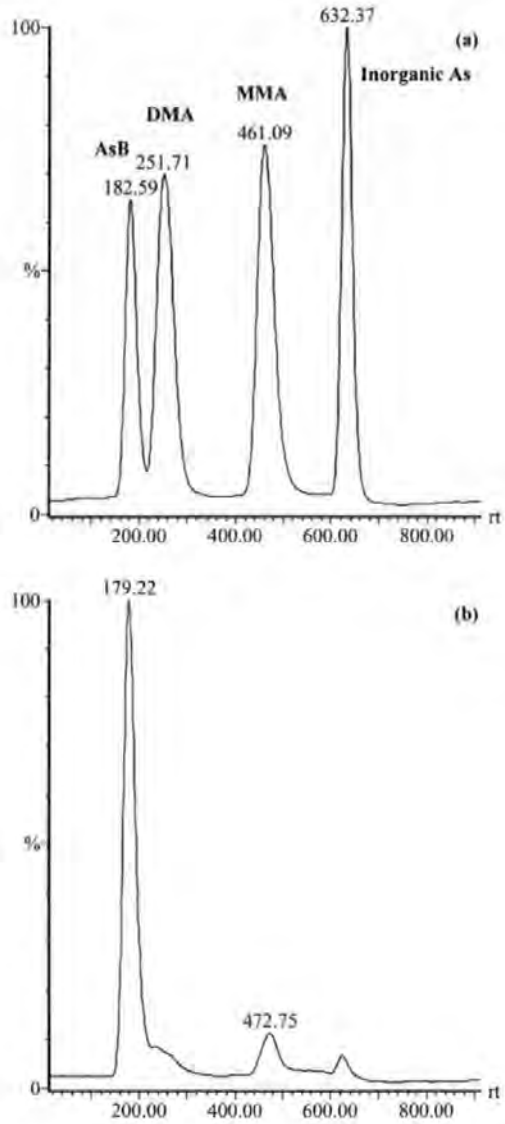


**Fig. 1** Chromatograms of arsenic species in aqueous solutions from biota studies; (a) Standard AsB, DMA, MMA and $As^{V}$. 200 μg $l^{-1}$ As in each species, (b) TORT 1.

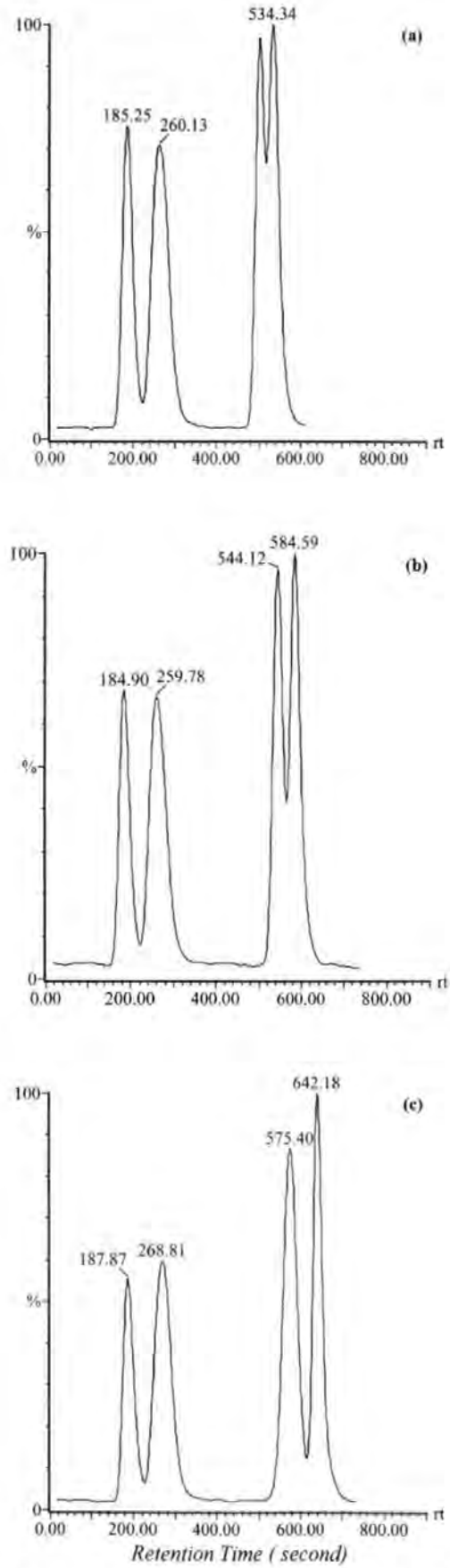


**Fig. 2** Chromatograms of AsB, DMA, MMA and $As^{V}$. 200 μg $l^{-1}$ As in aqueous solutions using different isocratic running times; (a) 180 s, (b) 240 s, (c) 300 s.

separation. The separation of AsB and DMA improved as the concentration of mobile phase **(a)** decreased but DMA took a long time to be eluted and the peak was affected adversely. The separation of MMA and inorganic As was not dependent upon the concentration of mobile phase **(b)** but upon the running time of mobile phase **(a)** before switching to mobile phase **(b)**. MMA is less ionic and therefore has less affinity for the stationary phase when compared with inorganic arsenic. Hence, the MMA is eluted earlier when the column is eluted with the mobile phase **(a)** and they are separated well as the running time of mobile phase **(a)** is increased, Fig. 2. The concentration of mobile phase **(b)** was selected as a compromise concentration so that MMA and inorganic As may be eluted in a short time period whilst ensuring that the concentration was not high enough to cause blockage of the ICP-MS sampling cone and torch by sodium salts from the mobile phase.

### 3.2 HPLC optimization for the phosphoric acid extracts of sediment samples

Fig. 3a shows the chromatogram of four standard arsenic species, $As^{III}$, DMA, MMA and $As^{V}$, respectively. This separation was achieved successfully using an anion-exchange stationary phase column. Advantage is again taken of the $pK_a$ values of the arsenic species to elute in the pH range 6–7.5 using the competitive phosphate anion. The analysis is

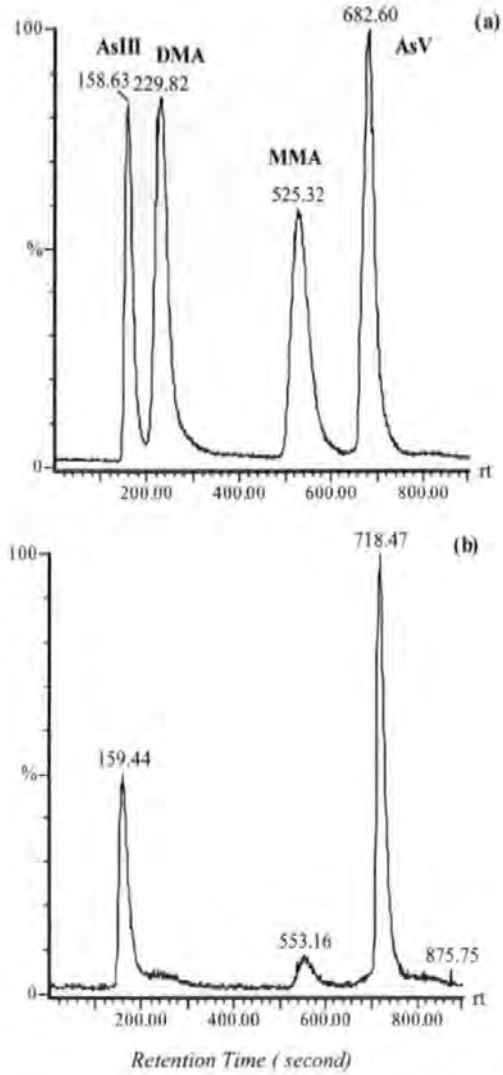


**Fig. 3** Chromatograms of arsenic species in aqueous solutions from sediment studies; (a) Standard $As^{III}$, DMA, MMA and $As^{V}$. 100 μg $l^{-1}$ As in each species; (b) River sediment extract.

complete in 900 seconds using two concentrations of mobile phase $H_3PO_4$, **(a)**: 2mM and **(b)**: 50mM (Table 4). $As^{III}$ and DMA are eluted with concentration **(a)** then MMA and $As^{V}$ are eluted when the mobile phase is switched to concentration **(b)**.

### 3.3 Reliability of the enzymatic extraction procedure for biota

Enzymatic extraction of samples using trypsin was chosen in this experiment for two important reasons; (i) it is known that trypsin is capable of extracting the As species without modifying them and (ii) it also has a high extraction efficiency. As part of the mass balance, total arsenic digestions using microwave digestion with nitric acid and hydrogen peroxide were performed. The total available arsenic concentrations are shown in Table 5 together with the measured species. High extraction efficiencies for As using trypsin were observed in every sample. A small reduction in the efficiency of As extraction from the swimming crab samples corresponds to the increase seen in the arsenic concentration in the residue. To validate the performance of the extraction procedure, it was necessary to determine total arsenic in the extracts of the CRMs DORM 1 and TORT 1 using $N_2$-ICP-MS. High recovery values of 98, 106 and 103%, compared with the total arsenic using acid digestion were found respectively. The integrity of As compounds in extracts is important as stated earlier. The limited range of arsenic compounds in CRMs is a major problem for speciation studies at present. One way to solve the problem is by comparing the results of As compounds in some CRMs that have been analysed by many groups of researchers using different extraction and detection techniques. The results are shown in Table 6. For DORM 1, AsB is the major arsenic compound found in this work with concentration of 15.4 μg $g^{-1}$. This is in good agreement with the literature concentration range of 14.5–16.5 μg $g^{-1}$. The only minor component found in this work is DMA which has also been found by Goessler *et al.*[16] with the same range of concentration. Values for MMA and inorganic As have been reported in some studies as less than limits of detection (less than 0.05 μg $g^{-1}$) or not detected and were not detected in this work. For TORT 1 the chromatogram is shown in Fig. 1b. Other studies have been found in the literature for a comparison to be made with the data obtained in this study. AsB is in good agreement as the major species with the concentration range 16.6–22.3 μg $g^{-1}$. Values for MMA and inorganic arsenic are detected as trace components in this work with a concentration of 2 and 0.6 μg $g^{-1}$, respectively. The ultra-trace compounds reported to be present in DORM 1 and TORT 1 in the literature vary widely. This is possibly because the concentrations are low and the techniques used were different. The high extraction efficiencies obtained in this work using trypsin on the CRMs (>98%) is in contrast to the lower efficiencies obtained using alternative extraction techniques.

### 3.4 Optimization and reliability of the phosphoric acid extraction procedure

The LGC 6137 estuarine sediment reference material was used as a sample in the optimization experiment. 0.5 g sample was extracted under various conditions as shown in Table 7 (the extraction process is shown in Table 2). The results show good extraction efficiency from 78–98% of total arsenic in extracts compared with the certified value. The conditions at 45 W power for 20 minute extraction were finally selected for the extraction in this work because they gave the highest efficiency with reduced risk of species modification, compared with a 20 minute extraction at 60 W. The stability of the arsenic species under these extraction conditions was also determined by spiking those arsenic species standards into the estuarine sediment reference material. The results show good stability for all the species after the extraction with a variability of less than 10% for $As^{III}$, $As^{V}$, MMA and DMA species. The most

**Table 5** Experimental results for the fish, crustacean samples and CRMs (μg $g^{-1}$ As dry mass), mean ± standard deviation ($n$ = 3)

| Sample | Total As[a] | AsB | DMA | MMA | Inorganic As | Total As in residue | Total As in extract | Efficiency of extraction[b] (%) |
|---|---|---|---|---|---|---|---|---|
| Sardine | 5.8 ± 0.4 | 4.5 ± 0.3 | 1 ± 0.04 | ND | 0.3 ± 0.01 | 0.6 ± 0.04 | 5.5 ± 0.6 | 95 |
| Catfish | 2.5 ± 0.3 | 1.9 ± 0.1 | 0.4 ± 0.03 | ND | 0.2 ± 0.01 | NL | 2.3 ± 0.2 | 92 |
| Tiger Prawn | 11 ± 0.5 | 9.7 ± 0.3 | 0.7 ± 0.01 | ND | 0.8 ± 0.01 | NL | 11 ± 0.5 | 102 |
| Swimming Crab | 17 ± 1.1 | 13 ± 1 | 2 ± 0.1 | ND | 0.9 ± 0.03 | 4.2 ± 0.1 | 14 ± 0.8 | 82 |
| DORM 1 | 17.4 ± 1 | 15.4 ± 0.6 | 0.6 ± 0.05 | ND | ND | NL | 17.1 ± 1.8 | 98 |
| TORT 1 | 22.1 ± 1.4 | 20.8 ± 1.4 | NP | 2 ± 0.1 | 0.6 ± 0.04 | NL | 23.4 ± 2.1 | 106 |

[a] Total arsenic concentration using acid digestion. [b] Efficiency of total As extraction compared with total arsenic using acid digestion. Concentrations of As in DORM 1 = 17.7 ± 2.1 and TORT 1 = 21.6 ± 1.8 mg $kg^{-1}$, ND = Not detectable; NL = No residue left; NP = Not performed.

**Table 6** Concentrations of arsenic compounds (μg $g^{-1}$ As dry mass) in DORM 1 and TORT 1 mean ± standard deviation ($n$ = 3)

| CRM | AsB | DMA | MMA | $As^{III}$ | $As^{V}$ | Extraction method | Analysis technique | Ref. |
|---|---|---|---|---|---|---|---|---|
| DORM 1 | 14.7 | NP | <0.03 | <0.05 | <0.05 | Chloroform-methanol-water | HPLC-ICP-MS | 20 |
| | 15.1 ± 0.6 | NP | NP | NP | NP | Chloroform-methanol-water | HPLC-ICP-MS | 14 |
| | 16.1 ± 0.4 | NP | NP | NP | NP | trypsin | HPLC-ICP-MS | 14 |
| | 16.5 ± 0.6 | NP | NP | NP | NP | methanol-water | HPLC-MO-HGAAS | 21 |
| | 14.5 ± 1.5 | ND | ND | ND | | trypsin | HPLC-MD-HGAAS | 22 |
| | 15.6 ± 0.7 | 0.49 | <0.03 | <0.03 | <0.03 | methanol-water | HPLC-ICP-MS | 16 |
| | 15.4 ± 0.6 | 0.6 ± 0.05 | ND | ND | | trypsin | HPLC-ICP-MS | This work |
| TORT 1 | 16.6 | 1.6 | <0.03 | <0.05 | <0.05 | Chloroform-methanol-water | HPLC-ICP-MS | 20 |
| | 22.3 ± 1.2 | ND | ND | 2 ± 0.2 | | trypsin | HPLC-MD-HGAAS | 22 |
| | 20.8 ± 1.4 | NP | 2 ± 0.1 | 0.6 ± 0.04 | | trypsin | HPLC-ICP-MS | This work |

ND = Not detectable; NP = Not performed; HPLC-MO-HGAAS = High Performance Liquid Chromatography-Microwave-assisted Oxidation-Hydride Generation Atomic Absorption Spectrophotometry; HPLC-MD-HGAAS = High Performance Liquid Chromatography-Microwave Digestion-Hydride Generation Atomic Absorption Spectrophotometry.

**Table 7** Optimization of phosphoric acid extraction for total available arsenic in LGC 6137 Estuarine sediment (12.4 ± 1.8 μg $g^{-1}$ As) ($n$ = 3)

| Power/Watts | Time/Minutes | Extraction efficiency (%) |
|---|---|---|
| 30 | 10 | 78–84 |
| 45 | 10 | 82–84 |
| 60 | 10 | 83–85 |
| 30 | 20 | 91–92 |
| 45 | 20 | 96–98 |
| 60 | 20 | 96–98 |

variable was found to be $As^{III}$ which decreased by 4–8% of the spiking value. A concordant increase in $As^{V}$ suggested that some $As^{III}$ was oxidised to $As^{V}$ during the extraction. A 1% increase and 2% decrease were found in MMA and DMA species, respectively which, considering the analytical uncertainty, was felt to be acceptable.

### 3.5 Arsenic speciation in samples from the Pak Pa-Nang Estuary and its catchment

Concentrations of total arsenic in fish and crustacean samples ranged from 2.5–17 μg $g^{-1}$ (Table 5). The concentrations found in this study are in the range that has been reported previously for various species of fish and crustacea.[23,24] In sardines and catfish which are free swimming feeder fish, lower concentrations were found of total arsenic (2.5–5.8 μg $g^{-1}$) than in the tiger prawn and swimming crab (11–17 μg $g^{-1}$), which are benthic feeding crustacea. A similar distribution of arsenic species was found in every fish and crustacean sample, where AsB is the major species with minor DMA and, more importantly, inorganic As (Fig. 4).

Concentrations of total arsenic in river and estuarine sediment samples range from 7–269 μg $g^{-1}$ and 4–20 μg $g^{-1}$ dry mass, respectively (Fig. 5). A high concentration, 269 μg $g^{-1}$, was found in the Pak Pa-Nang River near the mining area and this concentration decreased further away from the mining source. Arsenic in other rivers was found to have a decreasing gradient from the Bang Chak to Pak Nakorn Rivers. The lowest concentration was found in the Tha Sak river (4 μg $g^{-1}$) where there is no direct input of arsenic from the mining area (Fig. 5). In the estuarine sediments lower arsenic concentrations were found in the dry season compared with the wet season, with average concentrations of 10.4 ± 3.6 μg $g^{-1}$ and 16.2 ± 4.8 μg $g^{-1}$, respectively. $As^{V}$ was found to be the major species (*ca.* 90%) with smaller amounts of $As^{III}$ (up to 10%) in all the estuarine sediments. Only one organic arsenic species, MMA was found in some river sediments but this constituted not more than 2% of the total arsenic. The chromatograms of arsenic species in standards and extract of a river sediment are shown in Fig. 3. The high concentration of inorganic arsenic as shown in one example from a sampling station near the mining area (79 μg $g^{-1}$, Fig. 5) demonstrated the potential problem to human health and others organisms from arsenic storage. Sediment core samples show the temporal build up of the inorganic arsenic, similar in species distribution to the surface sediments. With an average total arsenic concentration of 63 ± 8.6 μg $g^{-1}$, down to 14 cm, the effects from the seasonal flood waters, carrying arsenic-loaded suspended particulate matter

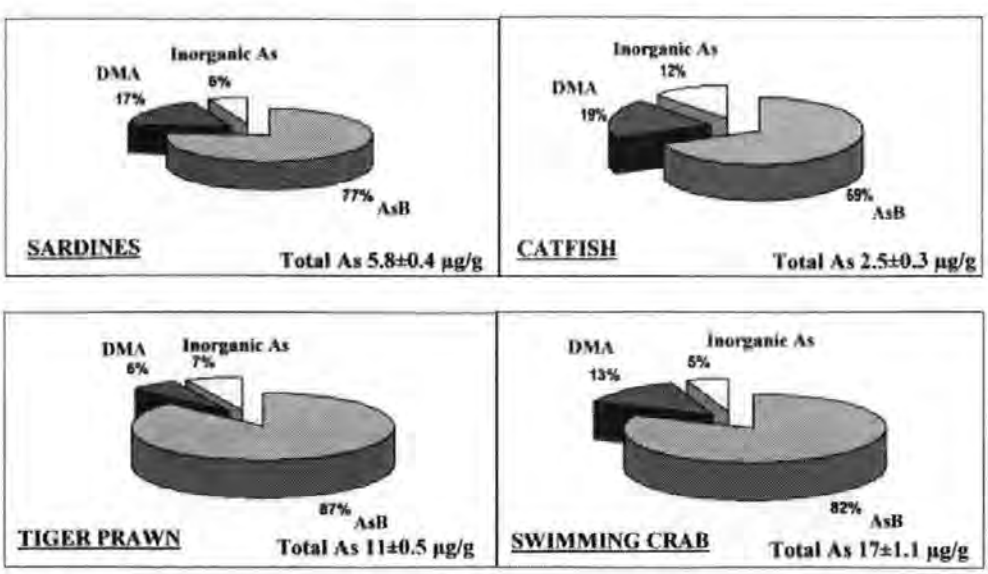


**Fig. 4** Arsenic in fauna ($n$ = 3); sardines, catfish, tiger prawn and swimming crab (dry weight basis).

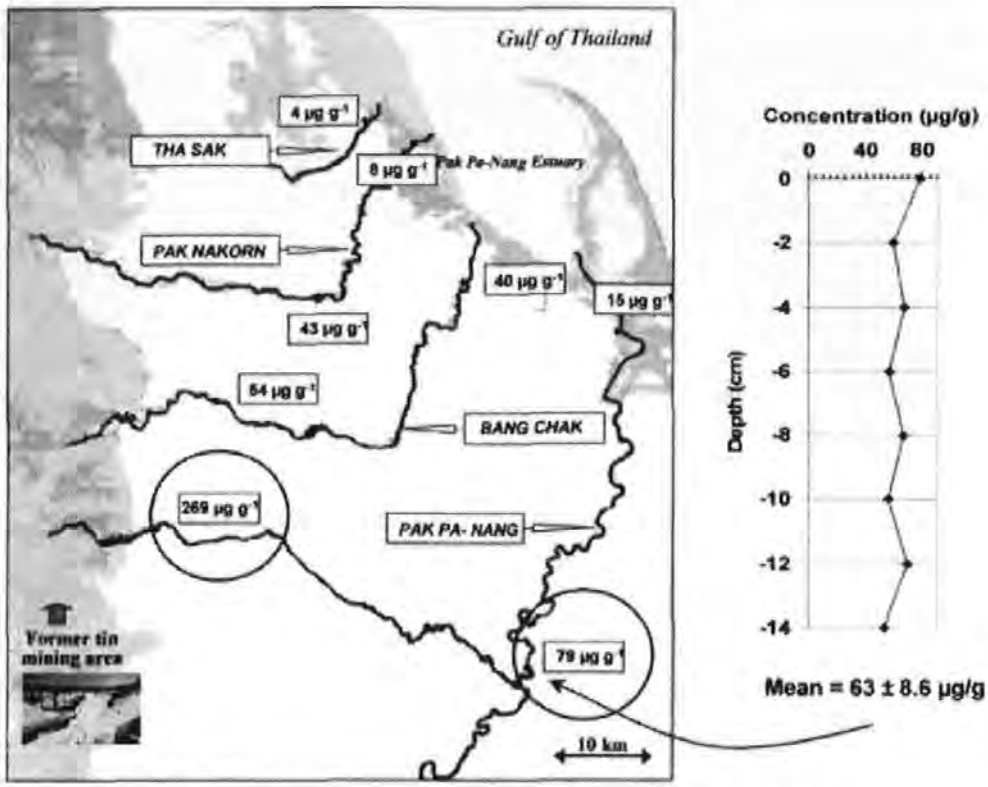


**Fig. 5** Arsenic concentrations in river sediment samples (dry weight basis).

within the water column from the catchment, are likely to continue for some time and be independent of mining activity. The continuing supply of the more toxic forms of arsenic in high concentration would appear to have contributed to the higher than normal levels of inorganic species found in the biota samples (up to 12% of total arsenic) from the bay itself.

## 4. Conclusions

Biota and sediment samples from the Pak Pa-Nang Estuary and its catchment in Southern Thailand have been analysed for their total arsenic content and arsenic speciation. Extraction methodologies based upon Trypsin enzymolysis and orthophosphoric acid solvation have been successfully applied to these biota and sediment samples respectively, resulting in high extraction efficiencies. Anion-exchange HPLC-ICP-MS was used to quantify species and little or no species modification was observed for these samples or from the CRMs employed as part of the validation procedure. The major species found in fish and crustacea was arsenobetaine with smaller levels of DMA and inorganic arsenic; the latter constituting 5 to 12% of the total arsenic. While the total arsenic content for biota ranged between 2.5 and 17 μg $g^{-1}$, that found in sediments from the estuary and rivers show a gradient increasing towards the mining area, up to *ca.* 270 μgAs $g^{-1}$. As the speciation of available arsenic from sediments is the most toxic arsenic forms (up to 90 : 10 $As^{V}$ ; $As^{III}$) the implications for human and other organisms' health must be considered. The potential is noted for a continued supply of toxic arsenic to the bay from periodic flood-water transport, which would elevate the contribution from these species to local edible biota. The higher levels of inorganic arsenic found in the benthic-feeding biota (crabs and prawns) indicate that quantities in excess of 0.5 kg (wet weight) would need to be ingested per day by an average Thai adult in order to approach the recommended threshold limit set by the WHO. At present, these biota are below the 1 μg inorganic arsenic $g^{-1}$ (wet weight) limit which is under consideration in the UK.


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