Report compiled by

Lara Luke BSc (Hons)Env Studs (Open)Dip Poll Con (Open)

For Scrutiny Hearing, 23rd April 2009.

Introduction:

In The Strategic Plan 2005-2010, under Strategic Aim Four, the States address the issue of contaminated land with the pledge to 'ensure that unpolluted air, clean water and uncontaminated land exist as a basic right for all'.

The Island Plan 2002 recognises that fishing for shellfish, wet fish and fish farming are important economic activities, which need safeguarding and supporting.

Good water quality in areas where shellfish live is essential to prevent contamination of shellfish. Bottom feeders, such as oysters and mussels, can be particularly sensitive to pollution and contaminates as they are filter-feeders (drawing water across their sievelike gills which can rapidly accumulate micro-organisms, heavy metals and organic contaminants).

The monitoring of the quality of shellfish waters helps to prevent them becoming contaminated, as there is a risk poor water quality could mean that shellfish become contaminated, which could then affect the people who eat them.

The situation we are faced with:

The excavation at the site of the incinerator is through material dumped at La Collette in its early phase when fly ash and bottom ash were mixed in with builder's rubble and other generally dumped items. The ash will have contained residue from electrical items and batteries etc with a high mercury content. (See section P 25 – 'Where is the ash from the incinerator?')

I trust that TTS would have the analysis of the soil which would have been prepared by the developers when they put in for the planning application for the construction of the energy from waste plant as the proposed development is built on landfill/reclaimed land which is considered to be contaminated. However was a fresh soil analysis completed after the addition of the contaminated land from Castle Quays?

After monitoring the progress of the construction process it has been noted that these excavated pits both fill with sea water and empty in accordance with the tides. As the tide comes in it is filtering through the porous outer walls and in reverse through to low tide.

Mr. Tony Legg, oyster farmer and marine biologist, adds this information on hydropneumatic erosion:

"Hydropneumatic Erosion: This process warrants most consideration. It is the process that leads to 'Blow Hole' formation on exposed cliff faces. Where wave action is severe, voids are formed within the eroded face. Subsequent wave action then compresses air within the void which can then expand explosively. Of interest is that this process can continue many tens of metres from the point of wave action and many meters above the high tide mark. (St. Catherine's Breakwater had a void 26 metres deep caused by this process, 25 km fetch). It may be that no area within the new reclamation is risk free from this process, including high level profiles."

What is the contaminated land likely to contain?

In the early 1990s, Jersey's Public Services Department, in response to concerns, commissioned Warren Spring Laboratory to undertake an analysis of emissions from the incinerator. Included was a detailed analysis of the ash produced by the incinerator. The table I, reproduced below is to be found on page 29 of the WSL report –

Table 1

TABLE 9 – CONCENTRATIONS OF METALS IN COMBINED RESIDUAL ASH

METAL	UNIT 2	UNIT 3
	μg g	μg g
Mercury	0.46	0.29
Vanadium	62.3	56.1
Chromium	212	244
Manganese	937	937
Cobalt	21.2	17.7
Nickel	60.3	74.9
Copper	18110	1160
Zinc	6200	4540
Arsenic	54.2	34.4
Selenium	1.41	0.53
Cadmium	33.5	34.0
Tin	274	272
Antimony	177	83.8
Thallium	0.45	0.33
Lead	4170	2020

Amongst the other information in the report are two tables – 11A and 11B – to be found on pages 30 and 31 of the WSL report. These two tables describe the 'CONCENTRATIONS OF DIOXINS AND DIBENZOFURANS IN COMBINED RESIDUE ASH", for units 2 and 3 respectively. These showed the total dioxin and dibenzofuran content of the combined ash from unit 2 to be 6.4112 ng-g, and a concentration of 0.5014 ng-g for unit 3.

Thus the WSL report demonstrates scientifically that the combined ash from the incinerator contains a variety of toxic heavy metals and polychlorinated compounds such as dioxins and furans which are a proven threat to human health. To further demonstrate this fact I reproduce below a section of a table titled "Toxic Effects of Common Hazardous Compounds". This is from page 476 of "Hazardous Wastes: Sources, Pathways, Receptors" by Richard J. Watts, published by John Wiley & Sons, ISBN 0-471-00238-0.

Table 2

Chemical	Acute Effects	Chronic Effects
Polychlorinated Biphenyls	Minimal acute toxicity	Chloracne; increased liver
	(0.5 g/Kg to 11.3Kg	enzymes; possible reproductive
PCB's		effects; act as cancer promoters
Dioxins and Furans	Chloracne, headaches,	Induction of microsomal
	peripheral neuropathy	enzymes; altered liver
PCDDs/PCDFs		metabolism; altered T-cell sub-
		sets; immunotoxicity; strongly
		implicated in carcinogenicity
		(may be a promoter)
Inorganic Compounds		
Arsenic	Loss of blood, intestinal	Myelogeneous leukaemia,
	injuries, acute respiratory	cancer of skin, lungs, lymph
	failure	glands, bladder, kidney,
		prostate and liver
Cadmium	Vomiting, cramping,	Oral ingestion results in renal
	weakness, and diarrhoea	necrosis and dysfunction;
		induces lung, prostate, kidney,
		and stomach cancer in animals;
		no documented human cancer
Hexavalent chromium	Readily absorbed by the skin	Lung cancer
	where it acts as an irritant	
	and immune-system	
	sensitizer; oral absorption	
7.5	results in acute renal failure	
Mercury	Central nervous system	Central nervous system
	impairment including injury	dysfunction, memory deficits,
	to motor neurons; renal	decrease in psychomotor skills,
NU-lea1	disjunction	tremors
Nickel	Not highly toxic, headache, shortness of breath	Immune system effects
	snortness of breath	resulting in allergic contact
		dermatitis

It appears that the WSL study did not test for polychlorinated biphenyls (PCBs) but it is likely that PCB is also present in the ash given its past use in electrical equipment. Only 5 of the 15 metal components of the ash identified in the WSL report are described in Table 2 so one must consider the fact of the existence of additional hazardous components in the ash to those listed in the table. (Source – proposition to the States by Senator Stuart Syvret, 10th June 2008)

Also important to note is that this analysis was compiled a number of years ago and since then, almost 20 years ago there has been a change in the type of waste that is incinerated. Think of the advancements in technology computers, mobile phones, i-pods, all of which are quite often updated and disposed of adding to the quantity of electrical waste. The same applies for televisions etc, plasma, LCD, all with their own disposal pollution aspects.

Definition of leachate:

This is the seepage of liquid through a waste disposal site, water passing through the ash/contaminated land.

Leaching:

Draft Planning Advice Note

Development of Potentially Contaminated Land - Guidance for Developers, Agents and Consultants

Extract from above guidance:

What chemical tests should I carry out?

Chemicals tested for should include all contaminants of concern. Contaminants suspected to be present on the site which are not part of a generic test suite (such as the ICRCL suite) should not be missed out of the analysis for this reason. You should also carry out leachability tests to assess how mobile a contaminant is and whether it may pose a risk to water resources. Laboratories used should preferably be UKAS (United Kingdom Accreditation Service) accredited for all tests on contaminants of concern which they carry out.

 $\underline{\text{http://www.gov.je/PlanningEnvironment/Environment/Environmental+Protection/Conta}\\ minated+land/Development+of+Potentially+Contaminated+Land.htm}$

Leaching means the removal of water of any soluble constituent from the soil/ ash or from waste tipped on land, therefore it is the gradual dissolution of a material from a solid containing it, e.g. metal from ore, heavy metal ions from ash etc.

How do we know heavy metals are leaching into available water on site?

We know this is happening as a result of two sets of water samples collected by the Environment Department. One for the application of a discharge permit, **Application Number DP(B)2009/03/01**, and the samples from the accident where one of the liners on the incinerator site was accidentally damaged by a site worker.

Below I reproduce my representation for the application of the discharge permit which outlines a number of concerns:

20th March 2009

Re: Application Number DP(B)2009/03/01

The points I make below are my representation to be taken into account with reference to the application for a discharge notice made by SBC Limited.

1. The salinity of the water.

Typical seawater has a salinity of 35 000 ppm of which 30 000 ppm is common salt (NaCl). Brackish water has a salinity range from approximately 1 000 ppm to 10 000 ppm.

Here we have a situation that has caused both seawater and rainwater to collect and mix together forming 'brackish' water.

In the application for the discharge permit there is no reference to the salinity of the water, which can be extremely detrimental to marine life as stated below:

Marine and river environments have obvious differences in water quality, namely salinity. Each species of aquatic plant and animal is adapted to survive in either marine, brackish, or freshwater environments. There are species that can tolerate both, but these species usually thrive best in a specific water environment. The discharge of brackish water into the surrounding waters, if done in large quantities and with any regularity, may alter the aquatic environment significantly. Fluctuations in salinity will result in changes in the community of animals and plants living in that location. It can cause either multiplication or disappearance of species causing an imbalance on ecosystems.

The salinity of the water must be established prior to discharging into the sea.

2. Retrospective permit:

How can you issue a discharge permit retrospectively, from the end of February 2009? With the high levels of toxic substances present in the tested water from the pit (as detailed in the application) that must have already been discharged, does that not mean that the sea has knowingly been polluted which is an offence under the Water Pollution (Jersey) Law, 2000?

3. Discharge rate:

With the two settlement tanks having a total capacity of 75 m3 and the maximum quantity that could be discharged in one day of 432 m3, that means that these tanks would have around 6 full water changes a day. How will these changes allow the silt to settle out? (Suspended solids in sewage would take around 2 hours to settle when stationary; however this will be a constant flow)

4. Oils.

How will oils be intercepted, as there is no explanation in the application?

5. Conductivity and hydrocarbon:

As the results of the analysis are not included with the application, when will they be available for the public to view, before or after the decision?

Where are the details for the hydrocarbon separator?

6. Application

Application is not filled out correctly, Part A not completed (it says all applicants); I could see no discharge points or sampling points clearly marked on the map; I can see no distance and depth relative to low water mark as the discharge is to coastal waters.

7. Temperature:

The maximum of the trade effluent to be discharged is 18°C, however where are the average sea temperatures, and what are the likely effects from the difference in temperatures on coastal waters of the Ramsar site?

8. Heavy Metals:

The presence of heavy metals in the sample of pit water would be there as a result of the seepage of water through the ash pit, collecting at the bottom, which is known as leachate.

'The lack of suitable inland disposal sites has meant that Jersey has increasingly turned to reclamation as the sole means of disposing of solid waste, with incineration playing an important role in reducing the volume of waste and prolonging the life of reclamation sites. However the possibility of leachate entering the marine environment is very real. If this occurred locally it would constitute a breach of the Dumping at Sea Law Sea Fisheries (Miscellaneous Provisions) 1974. This has led to the ash being dumped since 1987 above mean high water level at the reclamation sites.

When such leaching is excessive and/or prolonged, the metals can become concentrated up a marine food chain. Some bivalves, for example, concentrate certain metals tens of thousand times above the ambient level (Brooks and Rumsby, 1965). In extreme cases, potential public health risks arise because of the ingestion of contaminated seafood.

In Jersey the common limpet and brown fucoid seaweed have been sampled since 1994 at five locations (ie West of Albert, La Collette, St Aubin, Corbiere and Gorey). Samples have also been taken from Havre des Pas and Les Ecrehous.'

Source:

http://www.gov.je/Health/public_health/health_protection/pollution/Heavy+Metals.htm

Reading the above quote from the States of Jersey website, makes me question whether the application for a discharge permit is in fact legal as it states 'if this occurred locally it would constitute a breach of the Dumping at Sea Law Sea Fisheries (Miscellaneous Provisions) 1974'.

Why has mercury not been tested for?

As heavy metals bioaccumulate mercury would potentially have the highest adverse effect. Bacteria can change mecury in water to methylmercury which binds to the proteins in fish. The levels of mercury will be higher, the higher up the food chain you go. The main pathway of mercury to humans is through the food chain- fish. In all living things heavy metals accumulate and are stored quicker than they are broken down.

Out of the heavy metals tested for, lead, cadmium, arsenic and nickel are categorized as toxic substances. It is noted that antimony also has not been tested for.

Another omission in the category for substances undesirable in high amounts is nitrate, and phenols (highly toxic to living organisms, also can poison sewage treatment plants and taint water in very small concentrations).

Monitoring this discharge for heavy metals should not be allowed to cease if this discharge permit is granted.

The EEC has 13 Directives (1990) on water quality. Would this discharge comply with the Directives set down for shellfish in designated waters which require protection to make them suitable for fish life or to ensure that the quality of shellfish is suitable for human consumption?

Samples from pit:

Lead is 38.4 µg/l above Maximum Admissible Concentrations (MAC).

Iron 13,400 µg/l above MAC.

Manganese is 270 µg/l above MAC.

Are these levels safe to be entering our coastal bathing waters?

Testing for pH levels and treating will not affect the levels of heavy metals present.

How will the heavy metals be removed from the tanks?

9. Where are the baseline studies from the point of discharge? Also the water that is to be discharged to sea will need to have the same complete analysis as that done on the baseline seawater sample. This is to be able to make a meaningful comparison between the treated and untreated water. Baseline samples of the untreated seawater need to be taken as well at regular intervals.

10. In the 2007 Bathing Waters report (Jersey) it states that the quality of water is falling around this area by the number of coliform bacteria present, would the added problem of this discharge not cause the quality to further deteriorate? The report suggested that further testing for E-Coli should be commissioned as a result of the coliform count.

11. How will this discharge affect the large part of the Islands shellfish which is stored in viviers (seawater tanks) on the Victoria Pier, also the adjacent area to the west of La Collette (Elizabeth Castle) shellfish is also stored in nourrices (floating viviers) considering the high levels of heavy metals contained in the pit water?

12. Why is only the pH being continuously monitored?

Analysis of Waste Water

To determine the appropriate effluent treatment required and to monitor performance it is essential to test surface and process water prior to any discharge from source:

- After collection
- During treatment
- Prior to final discharge

Whilst some tests might be site specific, depending on the industry, it is essential to analyse for:

- Suspended lead
- Dissolved lead
- The acidity/alkalinity, i.e. the ph value
- Heavy metals such as antinomy, copper, zinc, arsenic, cadmium and mercury
- Test for oil, grease and dissolved salts, particularly if the process requires the use of caustic soda or other reagents that produce soluble heavy metal salts.

13. Will dioxins be tested for in both discharge and sea water?

Mussels have an important role in the circulation of dioxins. Mussels increase the net deposition of these substances on the sea bottom and make them more available for organisms living at the bottom. In addition mussels increase the residence time of the substances in the water masses and accumulate the substances and excreted them again.

14. Have organizations such as Ramsar, Save Our Shoreline and also the local fishing industry been consulted with regards to this application?

After reading, and giving careful consideration to the information contained within it, it is my opinion that this application has not given due consideration to the potential and highly likely adverse effects on the marine environment. There are not enough controls and remedies to prevent pollution of the sea from toxic substances.

Below is a copy of the lab report which was attached to the application:

Lab Ref: 90413 /

LABORATORY OF THE OFFICIAL ANALYST

SAMPLE SHEET

R	eceived from:	Camerons	La Carried
Joe Jest		Lnc	
0	Item:	Zx Water	SIE 20.
	FILE PRE	LIMINARY	REPORT
Heavy Metals	orianion/litical offi	ea Water	Pit Water
Lead My/L	_ Pb	22	88.4
Zine Myl		<10	100
Copper Mg/		<10	40
Iron Mg		120	13,600
Manganese 1		<10	320
Asseric 1		25	13.4
Chronium		< 7	12.8
Cadmin	•	< 0.5	< 0.5

With reference to the other issue of the accident and the water in-flow and trench, I have reproduced the correspondence about the incident:

25 March 2009 01:17 Dear William.

I would like to know who the 'regulator' is that took the samples for testing from the initial excavation 'accident' that this application has arisen from. Where are these samples being analysed and on who's behalf? Also could you tell me the scope of the testing and when these test results will be available for public reading?

I would like to thank you in advance for your time and help.

Best Wishes, Lara Luke

Thu, 26 Mar 2009 17:59:19 +0000 Dear Lara,

Thank you for your email. To answer your question regarding the Regulator - that is me, and my team - Environmental Protection, under delegated powers from the Minister for Planning and Environment. We regulate a variety of environmental legislation, the most pertinent Laws in this case being the Waste Management (Jersey) Law 2005 and the Water Pollution (Jersey) Law 2000.

We took 2 samples (one from the inflow and one from the ponded liquid) from liquids entering a trench at La Collette which had been excavated beside a hydraulically independent engineered cell containing ash from the incinerator.

I think it is important at this stage to avoid confusion, to advise you that the samples we took from this location are unrelated to the application made to discharge brackish waters from the site. The application has been made to facilitate the construction of the new EFW Facility and has no relevance to the trench samples.

The samples were analysed at the States Analyst to aid our understanding of the likely contents and the results are as follows.

Determinand	13/03/09/WP/001	13/03/09/WP/002
As	25	33
Cd	0.6	1.8

Cr	2	28
Cu	10	60
Hg	results to follow	
Ni	60	80
Pb	12	97
Se	results to follow	
Zn	20	140
рН	8.41	10.32

Note: Analysis - Total metals in micrograms per litre.

I attach a report that we put together at the time for your interest and would advise that we continue to work with Transport and Technical services to ensure that proposed remedial works are to our satisfaction. Might I propose that we meet to discuss any issues you have in respect of both the trench excavation issue and the discharge consent application. I would be happy to hear from you to arrange this.

I hope this is of assistance to you,

Yours sincerely,

William Peggie
Assistant Director - Environmental Protection

Report attached to above email:

POLLUTION REPORT 2009022 – LA COLLETTE SERVICE TRENCH EXCAVATION – 13 MARCH 2009.

Page 1 of 5

EXECUTIVE SUMMARY:

On Friday 13th March at 17.00, Environmental Protection (EP) were called by Transport and Technical Services (TTS) staff who advised them that there had been an escape of liquid from the direction of an Ash cell on site at La Collette into a newly excavated service trench. They were concerned about the possibility of the leak worsening and any potential impact the situation may have on surrounding controlled waters, specifically the Ramsar site area to the east of the reclamation

On attendance at the site EP determined that there was no visible pollution to the marine environment which was some distance away and that seepage from the direction of the ash cell was contained either in the trench or in the surrounding soils

Responsibility now lies with TTS and their contractors to keep a watching brief on the situation and to provide a remediation plan which must be agreed with Environmental Protection prior to commencement of those works. EP Officers attended in their capacity as regulators of the Waste Management (Jersey) Law 2005 and the Water Pollution (Jersey) Law 2000. During the course of the site visits, it became apparent that no pollution to controlled waters or breaches of the Waste Management (Jersey) Law 2005 had occurred hence the generation of this report as EP do not intend to pursue any formal action at this time. This situation may change in future if there is any change in the site circumstances.

BACKGROUND

On Friday 13th March a TTS sub contractor on EFW groundwork was reported to have excavated through the edge of a cell containment membrane whist digging a service trench along the northern edge of the La Collette site between the Jersey Electricity compound and an area of historic ash disposal.

It was further reported that a small quantity of potentially contaminated water had leaked into the trench. This leak had reportedly been ongoing for between two to five hours and there appeared to be, at the time of the site visit, in the region of six to seven bucketfuls of liquid in the bottom of the trench. See Photo 1.

The liquid was seeping from the cell-side wall of the trench in a central section of the excavation and the rate of flow was estimated by TTS at the time to be 6 litres per minute though this was not measured or substantiated. The water ponded in the trench and at one end for a matter of seconds appeared to drain beneath the base to the surrounding soil. To stem this flow the contractors used a shovel to create a dam with existing soil from the base of the trench and cement which was added to solidify the mix. See Photos 2 and 3:

Environmental Protection officers took 2 samples, reference nos: 13/03/09/WP/001 and 13/03/09/WP/002. These were delivered to the States Analyst on 16 March 2009 for analysis of pH and a suite of metals. Results are expected within a week.

The actions taken to stem the flow of liquid were firstly to shore the edge of the excavation with timber: See Photo 4. and then to apply suction to the cell via a riser pipe (integral to the construction of the cell) by vacuum tanker: See Photo 5. The application of suction immediately resulted in the cessation of the seepage of liquid to the trench. It was agreed in discussions on site between EP, TTS, and TTS Contractors that more liquid would be removed in this manner during the course of the evening to ensure that levels in the cell did not get to the point that would result in further seepage. EP Officers requested that the site Project Manager called to update on the situation the following day. A call was received at 09.50 from him to report that a further 4 loads of liquid had been removed during the course of Friday evening and that on Saturday morning there was no evidence of seepage to the trench.

POLLUTION REPORT 2009022 – LA COLLETTE SERVICE TRENCH EXCAVATION – 13 MARCH 2009.

Page 2 of 5

CURRENT SITUATION:

An officer attended site on the afternoon of 16 March and confirmed that the situation had not changed and that there was no water in the trench. Discussions have been held between EP and TTS and a remediation plan has been requested from TTS prior to any remedial works or further project works on this section of excavation taking place. Within this an explanation must be provided in respect of how and why the breach of the liner occurred and explaining how TTS propose to prevent a reoccurrence. The plan should also describe in detail how TTS propose to repair the liner and reengineer the cell to provide containment of potential pollutants in the ash cell. They should finally indicate when this will take place and what timescales are proposed. This report has been requested verbally and TTS have agreed to supply.

WP. 16/3/09

POLLUTION REPORT 2009022 – LA COLLETTE SERVICE TRENCH EXCAVATION – 13 MARCH 2009.

Page 3 of 5

Photo 1

Photo 2

POLLUTION REPORT 2009022 – LA COLLETTE SERVICE TRENCH EXCAVATION – 13 MARCH 2009.

Page 4 of 5

Photo 3

Photo 4

POLLUTION REPORT 2009022 – LA COLLETTE SERVICE TRENCH EXCAVATION – 13 MARCH 2009.

Page 5 of 5

Photo 5

Sun, 29 Mar 2009 03:28:44 +0100 Dear William,

I would like to thank you for your detailed reply.

After reading through all the information that you have given me I do have a couple more questions for you:

- Which sample was taken from where, this is not indicated. I assume that you and your team would have got to the incident as soon as you could, and would have taken the samples on arrival. The concerning aspect of this is that it is the same water, in-flow and trench, therefore one would assume similar results for heavy metals. However these samples highlight a remarkable difference. Why could this be? The only other place this 'same' water has been is in the trench, so either these metals were absorbed into the water extremely quickly or the soil is highly contaminated. I trust that TTS would have the analysis of the soil which would have been prepared by the developers when they put in for the planning application for the construction of the energy from waste plant as the proposed development is built on landfill which is considered to be contaminated. Do you have a report on the analysis of the soil on the site?
- Do you have the results for mercury and selenium levels yet?
- Am I able to have a copy of the remediation plan?
- Are tidal waters entering the site at any point? If the sea was entering the site, isn't it
 highly likely that controlled waters would be at a high risk of being polluted especially by heavy
 metals?
- Where has the water come from?
- Has the salinity been tested?
- Is it possible that other areas of the ash pit membrane's could be damaged causing leakage?

I would like to thank you in advance for answering my questions.

Yours Faithfully,

Lara Luke

I am currently waiting for a response to this email.

From the samples that I have been able to see, they indicate that there is a problem with the leaching of heavy metals into the available water, which therefore indicates that the same leaching effect will be happening when the tidal flow both enters and leaves the excavated site. This leaves the surrounding controlled waters as the receptor for pollution.

What are the pathways for pollutants to enter the sea?

There are two main pathways, point source and non-point source.

Non-point source:

There's a type of pollution that degrades bodies of water and it's called nonpoint source (NPS) pollution because it doesn't come from a single source, or point, such as a sewage treatment plant or an industrial discharge pipe.

NPS pollution occurs mainly through storm water runoff. When it rains, runoff from farmland, city streets, construction sites, and suburban lawns, roofs and driveways enters

our waterways. This runoff often contains harmful substances such as toxics, excess nutrients and sediments. NPS pollutions effects seldom show up overnight -- they often go unnoticed for years. This characteristic makes it all the more difficult to control. There are four major forms of NPS pollution: sediments, nutrients, toxic substances and pathogens.

Sediments - are soil particles carried by rainwater into streams, lakes, rivers and bays. By volume, sediment is the greatest pollutant of all. It's caused mainly by erosion resulting from bare land, poor farming practices, and construction and development.

Nutrients – are substances which help plants and animals live and grow. NPS officials are most concerned about excessive amounts of two nutrients; nitrogen and phosphorus.

Fertilizer and animal waste are the main sources of these substances.

Toxic substances - are chemicals which cause human and wildlife health problems. They include organic and inorganic chemicals and metals, pesticides, formaldehyde, household chemicals, gasoline, motor oil, battery acid, roadway salt and so on.

Pathogens - are disease-causing microorganisms present in human and animal waste. Most pathogens are bacteria.

Point source:

This is direct discharge of a pollutant(s) into a body of water, which can be directly linked, to for example a pipe.

Applying this to the current situation we have the nonpoint pathway, through the outer walls of the reclamation site, and through the infill and back out in to the sea, and also the possibility of 'treated' water being discharged at point source.

Life in bays could not exist without nutrients, but too much of a good thing often causes more harm than good. Nutrients over-enrich our waterways causing algal blooms which deplete oxygen. This makes the oxygen unavailable to fish and shellfish so they suffocate and die. The algae also cloud the water and coat underwater vegetation, cutting much needed sunlight. Sediment clouds water too, and it obstructs waterways, clogs sewers, interferes with navigation, and smothers fish and shellfish spawning grounds. Natural erosion and sedimentation occur at a lower rate than that resulting from human land use activities.

Underwater plants and aquatic animals are particularly threatened by NPS pollution. Oysters, shad, herring, striped bass and submerged aquatic vegetation -- considered by many to be the foundation of a stable aquatic ecosystem -- are damaged by this pollution.

Was this potential hazard identified in the Environmental Impact Assessment for the proposed incinerator?

Yes, please read extract from the EIS:

16.3 Impacts and Potentially Polluting Processes During Construction 16.3.1 Source Pathway Receptor Analysis

The following considerations apply to the proposed Energy from Waste facility:

• Eno rivers or streams would be affected by the proposed facility:

- The existing ditches, drains and settlement lagoons would either be used for the settlement of surface runoff or replaced with similar facilities during the construction period;
- Emechanisms to prevent runoff during construction including fuel tank bunding, settlement lagoons etc,
- •€no private water supplies, surface water or borehole abstractions would be affected; and
- The works would not increase the risk of flooding.

The potential sources of pollution to coastal waters would be both during construction and operation of the site. The potential pathway is infiltration into the fill and then dispersal due to tidal movements affecting intrusion of water under the fill area. At the detailed design stage trial pits and boreholes would be included as part of the ground investigation in order to allow testing for contaminants and determine the exact nature and depth of the fill in the area of the proposed foundation works. It is proposed that a "watching brief" for contaminated or hazardous materials should be adopted during the site development and construction phases. Any visual and olfactory evidence of contaminated or hazardous materials will result in further investigations being undertaken which may include laboratory testing and the material suitably dealt with by measures which may include containment or in exceptional circumstances disposal of the Island. The main receptor is the coastal waters which are designated as the South East Coast of Jersey RAMSAR Site, due to its high ecological value and diversity of habitats. This RAMSAR site would potentially be vulnerable should pollutants be released during construction or operation of the site. Groundwater beneath the side is also vulnerable but it is assumed for this assessment that it is in hydraulic continuity with the sea. The design of the facility would aim to break the links between sources of pollution during construction and operation and the receptor which is the coastal RAMSAR site.

The potential hazards have been identified but no risk assessment has been properly conducted. There is no application of the precautionary principle, in fact other than stating what could happen, and has, no remediation plan has been considered or discussed in the environmental impact statement.

This is a breach of the Planning and Building (Jersey) Law 2002; please see Appendix 1 for the breakdown of the law.

It concerns me that in the EIA, it mentions that the existing drains may be used for surface run off etc. Given the level of contamination in the area, this could potentially also pollute our sewerage system. In some older landfills, leachate was directed to the sewers but this can cause a number of problems. Toxic metals from the leachate passing through the sewage treatment plant concentrate in the sewage sludge making it difficult or dangerous to dispose of to land without incurring a risk to the environment.

As sewage treatment works discharges are being improved throughout Europe and many other countries, the sewage treatment works operators are finding that leachates are difficult waste streams to treat because they contain very high ammoniacal nitrogen concentrations, they are usually very acidic, they are often anoxic and, if received in large volumes relative to the incoming sewage flow, the lack of Phosphorus in particular can result in nutrient starvation for the biological communities that perform the sewage treatment processes making leachate a difficult to treat waste stream. However, within aging municipal solid waste landfills this may not be a problem as the pH returns close to neutral after the initial stage of acidogenic leachate decomposition. Also many older leachate streams also contained a variety of synthetic organic species and their decomposition products, some of which had the potential to be acutely damaging to the environment.

Where is the ash from the incinerator?

In many respects this is a difficult question to answer. Before 1995, the incinerator ash (bottom and fly ash) was simply dumped along with inert waste in the West of Albert site, therefore it could be anywhere and in fact parts have been excavated and have now been transported to the La Collette Phase II site to various locations. Since 1995 the ash

was required to be placed in lined pits. However in the early days no records were kept of the position of the pits. (See Appendix 2 – Answers to Senator Stuart Syvret's proposition, question vii).

The excavation at the site of the incinerator is through material dumped at La Collette in its early phase when fly ash and bottom ash were mixed in with builder's rubble and other generally dumped items. The ash will have contained residue from electrical items and batteries etc with a high mercury content.

Even though since 1995 separate ash pits were created they actually "sit' upon material as previously described and the sea is constantly washing below this material. Now of course the sea action is made worse by intrusion and opening the voids/fissures etc. The sea is now directly available to all levels of the fill and not just the bottom. The leaching rate will have increased as a result. Also these pits are lined with strengthened polythene sheeting which is overlapped and held in place with tyres. This type of construction would not prevent the leaching of various pollutants down through these pits from rain water etc and also would not prevent tidal surges from entering them from below. This leachate would be then flushed into the surrounding controlled waters. This does not take in to account any damage that may have occurred to the liners, whereby it would exacerbate the problem.

Former States Medical Officer of Health, Dr. John Harvey, and Health Protection Officer, Steve Smith, co-authored a report titled: 'Health Impact of the West of Albert Pier Reclamation Site.' Below is a quote from the report compiled in 2001 –

"Recent published studies have recognised the possible cumulative and synergistic effect of multiple hazardous agents, and have looked at the effect of exposure to hazardous sites, not individual toxins. The exposure risk is residence near to contaminated sites.

"The risk of adverse birth outcomes has been the focus of two such studies. A study of all residence near landfill sites in Great Britain showed small excess risks (c.10-

20%) of some congenital anomalies and low birth weight. This was not greater near sites with special waste (i.e. known toxic waste such as incinerator ash) possibly because these sites were subject to strict regulation. The authors noted that the small excess could be due to residual confounding (unmeasured effect of deprivation) or data artefacts. Another Europe wide study showed higher levels of risk for congenital anomalies. This study, known as EUROHAZCON used data from 7 registers in 5 countries. It showed an increased risk (2 – 3 times higher) for mothers living within 3km of landfill sites.

"A study in Canada showed increased risks of certain cancers for men living near solid waste landfill sites. The increases of twice the risk were shown for cancer of the pancreas in men living within 1.25km, cancer of the liver for those living within 1.5km, and non-Hodgkin's lymphoma within 2km."

Some 8 years ago health effects were being acknowledged by the States, why with today's knowledge and technology are there no proper controls on dealing with waste such as incinerator ash and its safe disposal?

Chemical pollution of the Aquatic Environment by Priority Pollutants

Any chemical in high enough concentrations can become a pollutant; however there are some chemicals that can be selected as being high priority for control in the aquatic environment because of their capability of exerting adverse effects even in low concentrations.

The 'red list' is a list of 23 dangerous substances, designated in the UK whose discharges to water should be minimised whilst using the BATNEEC (best available technology not entailing excessive costs) principle.

Red list substances	Additional substances on priority list
Mercury and its compounds	Copper
Cadium and its compounds	Zinc
Gamma-hexachlorocyclohexane	Lead
DDT	Arsenic
Pentachlorophenol	Chromium
Hexachlorobenzene	Nickel
Hexachlorobutadiene	Chloroform
Aldrin	Carbon tetrachloride
Dieldrin	Azinphos-ethyl
Endrin	Fenthion
Polychlorinated biphenyls	Parathion
Dichlorvos	Parathion-methyl
1,2-Dichlorethane	Trichloroethylene
Trichlorobenzene	Tetrachloroethylene
Atrazine	Trichloroethane
Simazine	Dioxins
Tributyltin compounds	
Triphenyltin compounds	
Trifluralin	
Fenitrothion	
Azinphos-methyl	
Malathion	
Endosulphan	

If extra background information on UK legislation for chemicals, please visit the website below:

 $\frac{http://www.ukooaenvironmentallegislation.co.uk/Contents/topic_files/offshore/Productio}{n_chemicals.htm}$

SEPA discharge consents

SEPA is responsible for the regulation of discharges to controlled waters under Part II of the Control of Pollution Act 1974 (as amended) and for the granting of consents and service of instruments to discharge under that Act. It inherited these functions under the Environment Act 1995 from the river purification authorities.

Since its inception, SEPA has been formulating policies to discharge these responsibilities, including :

Consenting policy for discharges to controlled waters (SEPA Policy No. 3, Version 1, July 1996) (draft);

Microbiological standards in marine waters (excluding shellfish waters) in relation to design criteria for discharges (SEPA Policy No. 27, Version 1, September 1998);

Initial dilution and mixing zones for discharges from coastal and estuarine outfalls (SEPA Policy No. 28, Version 1, September 1998).

Point source discharges for which consents are required include sewage and industrial discharges from non-prescribed processes (trade effluent). Prescribed processes require an IPC authorisation which is also issued by SEPA. Discharges from marine fish farm installations are considered to be trade effluent and require a discharge consent.

The procedure for the application for a discharge consent and the form of consent conditions (95 percentiles, upper tiers and absolute limits) is similar to that in England and Wales.

There is a requirement for all existing discharges to meet the following statutory requirements:

EC Bathing Waters Directive and UK Regulations (appropriate mandatory standards must be met at designated bathing waters);

EC Urban Waste Water Treatment Directive and UK Regulations (the appropriate level of treatment should be applied to the sewage, based on the population equivalent of the agglomeration served by the sewerage system);

EC Dangerous Substances Directive and UK Regulations (EQSs for List I and II substances in receiving waters must be met, including standstill provisions);

EC Shellfish Directive and UK Regulations (appropriate standards must be met for designated shellfish waters).

North Sea Conference and OSPAR commitments (reduction in loads of toxic substances in discharges to the marine environment must be demonstrated).

SEPA=s policy on initial dilution and mixing zones (SEPA Policy No 28) sets out requirements to be met in the design of new or modified discharges. For discharges with greater than 100 population equivalent, outfalls should be designed and constructed to provide the following minimum initial dilution to reduce both the visibility of density slicks and the occurrence of smell nuisance to acceptable levels:

minimum initial dilution of 100 times (95 percentile) for primary treated effluent; minimum initial dilution of 50 times (95 percentile) for secondary treated effluent, including septic effluent;

minimum initial dilution of 50 times (95 percentile) for significant new or modified industrial discharges (to be judged on an individual basis).

Modelling studies are required to determine the best location and design of the outfall and to demonstrate achievement of the minimum initial dilution requirements. SEPA specifies the requirements for modelling studies and recommends the use of one of 3 models: ELSID, PLUMES or CORMIX (see SEPA Policy No. 28).

Requirements for mixing zones are also specified to inform the design of an outfall and the consent conditions to be set to achieve compliance with statutory requirements and to protect the environment. These requirements include:

a limitation on the size of a mixing zone to 100 m in any direction; UK or SEPA Environmental Quality Standards (EQSs) should not be breached outwith the mixing zone;

where toxicity-based criteria are used, there should be no residual toxicity outwith the mixing zone;

neighbouring mixing zones should not merge and ideally should be at least 100 m apart;

no mixing zone should impinge on the Mean Low Water Springs (MLWS) shoreline:

no mixing zone should plug an estuary, sea loch or small bay;

the mixing zone will not be allowed to jeopardise the integrity of any European marine site:

the mixing zone should not give rise to significant slicks or other aesthetic problems;

accumulation of solids on the sea bed must not threaten the achievement of standstill clauses for List I substances outwith the mixing zone and not cause acute toxic effects to sediment-dwelling organisms within the mixing zone.

SEPA has developed a policy for microbiological standards in marine waters (except shellfish waters) in relation to design criteria for discharges (SEPA policy No 27). This policy has some influence on all marine waters in Scotland.

For all marine waters, no new or modified discharges will be allowed to result in deterioration of the class which is currently achieved under the coastal classification scheme nor threaten progress in improving class C and D marine waters identified in SEPA's corporate plans.

For identified bathing waters, existing discharges must enable mandatory standards to be achieved and, for new or modified discharges, the outfalls must be designed so as to achieve guideline standards.

SEPA may also designate >recreational waters= where significant water contact activities are practised outwith identified bathing waters. SEPA will require mandatory microbiological standards to be achieved at relevant times of year and promote the achievement of guideline standards where appropriate.

SEPA will also adopt a strong presumption that mandatory microbiological standards are achieved at 'shoreline waters' (i.e. those visited by the public).

As in England and Wales, the primary driver for the derivation of consent conditions is compliance with statutory requirements, in particular bathing water, Urban Waste Water Treatment and shellfish water standards. SEPA has supplemented these requirements in Scotland to some extent with their policies. These policies enable Scottish Natural Heritage (SNH) to verify that consent conditions for new or modified discharges in or close to European marine sites have been set according to the procedures outlined in these documents.

The approach to the regulation and monitoring of cage fish farming in Scotland has been comprehensively laid out in a manual of procedures (SEPA 1998). This manual provides details of the application process, the assessment of the application, setting consent limits, process for granting and refusing a consent, monitoring, data management and use of information and review of consents. Conservation agency staff should have access to this document to ensure that all cage fish farming consents have been set according to the process.

Source: http://www.ukmarinesac.org.uk/activities/water-quality/wq2_2_1.htm

General Information on shellfish:

The shellfish family is composed of gastropods, with a single-piece shell (sea snails, whelks, barnacles, abalone, etc.) and bivalves, with a shell made of 2 distinct parts (oysters, mussels, clams, scallops, etc.).

Shellfish have an especially high mineral element content (calcium, magnesium) and oligo-elements (zinc, iron, iodine, selenium). They are also high in certain vitamins, like vitamin B, vitamin D and vitamin E. Shellfish are low in calories (60 to 80 KCAL/100g of meat), high in protein (10 to 15 g/100 g) and low in fat (1 to 2 g/100 g). Like fish, they contain lipids made up primarily of fatty acids that are good for the cardiovascular system. Their cholesterol content is on par with that of meats (50 to 80 mg/100g).

Shellfish can concentrate up to 100 times the bacterial and virus content present in seawater, thereby explaining why it can be harmful to your health to eat shellfish from polluted waters. They also contain high concentrations of toxic plankton, chemical

compounds such as heavy metals (lead, mercury, cadmium) and certain organic compounds (hydrocarbons, polychlorobiphenyls – PCBs, tributyl tin – TBT, certain pesticides, etc.).

Long-term classification of shellfish harvesting areas:

Classification of areas (Class A, B, C or prohibited)

As with previous hygiene legislation, under the new Hygiene Regulations European Union Member States are still required to put in place a programme for monitoring and classifying shellfish harvesting areas. Production areas are categorised by the level of microbiological contamination, namely the level of E. coli contamination found in shellfish sampled from a site. These areas are classified as Class A, B, C or prohibited: Class A - shellfish contain less than 230 E. coli per 100 grams of flesh Class B - shellfish contain less than 4,600 E. coli per 100 grams of flesh Class C - shellfish contain less than 46,000 E. coli per 100 grams of flesh Prohibited area - above 46,000 E. coli per 100 grams of flesh (Information from the Food Standards Agency)

It appears from the recent press release of Dr. Rosemary Geller, that she has omitted a class – Prohibited area.

States working together with fishermen to ensure safety of local shellfish

Press Release 1st April 2009

Over the last years there has been a change in the classification of some of shellfish harvesting areas in Jersey . This means that products need to be relayed or purified before they can be marketed for human consumption.

The actual cause of this remains unknown but is consistent with the trends in the UK and other European countries. It has been noted that there has been a change in weather patterns resulting in unusually heavy rainfall and this may be correlated with impacts on beach areas and the shellfish farms.

States officials for Health, Environment and Transport and Technical Services have been working together with the industry to try to identify any possible issues that could be impacting on local shellfish farms and a large scale investigation continues to be carried out.

The local shellfish farmers routinely provide samples for classification purposes and for the research work that is being undertaken.

The classification of the areas is based on food safety standards for E coli and all business operations are approved under European food regulations.

Dr Rosemary Geller, Medical Officer of Health said:

"We are committed to supporting the shellfish industry in identifying any possible impacts on harvesting areas. A range of sampling techniques has been employed but this is a complex issue and it will take some time to complete the research. My officers are working closely with the businesses affected and are putting appropriate public health measures in place so that the public can be confident that any products marketed for human consumption meet food safety standards."

Food law across Europe, defines raw oysters as a 'high risk food', and as such they require strict food hygiene controls. The UK Food Standards Agency recommends that raw oysters can be cooked to improve their safety.

Notes

The classification of harvesting areas is based on a food safety standard for E coli and is determined on an annual basis following a minimum of 12 monthly samples. **Class A** – less than 230 E coli/100g flesh – product can be eaten raw

 ${f Class~B}$ – 231-4,600 E coli/100g flesh – the product must be purified, relayed or cooked to meet class A standards

Class C – over 4,600 – product must be relayed purified, cooked to meet Class A standard.

'High risk foods' are those that support the growth of pathogenic microorganisms and are ready to eat.

Bathing water:

Other problems with sea water:

Assessment of bathing water quality for the States of Jersey 2007

A Report to
Environmental Protection, States of Jersey
Dr Mark Wyer
Professor David Kay
February 2008
Final Report

1.3 Compliance assessment

Table 1.1 lists the raw data for bacterial concentrations at each location.

Concentrations were compared with the following *Imperative* or *Guide* values specified in Directive 76/160/EEC:

Standard

Organism *Imperative Guide*

Total coliform 10,000 cfu/100ml 500 cfu/100ml Faecal coliform 2,000 cfu/100ml 100 cfu/100ml

Faecal streptococci -- 100 cfu/100ml

Any results exceeding these values are highlighted in Table 1.1, bold figures indicating *Guideline* exceedence and bold/underlined figures *Imperative* exceedence. The Directive specifies that 95% of samples (i.e. nineteen samples in twenty) must show bacterial counts below the *Imperative* criteria defined for total and faecal coliforms. The *Guide* Standards for the two coliform parameters require 80% of samples (i.e. sixteen samples in twenty) not to exceed the specified values. In the case 2 of faecal streptococci, the *Guide* criterion can be exceeded in 10% of samples (i.e. two in twenty samples) through the bathing season. Temporal plots of water quality at each location, along with corresponding daily rainfall, are shown in Figures 1.2 and 1.3. Any values above or below the respective upper and lower limits of detection have been plotted as the detection limit values in these plots. The proportions (%) of samples complying with the EC Directive criteria for bacterial parameters at each location are listed in Table 1.2.

All sixteen of the bathing water monitoring locations complied with the *Imperative* standards. A further summary of the compliance of Jersey bathing waters with EC standards is given in Figures 1.4 to 1.6, which provide maps showing compliance for each of the three faecal indicator organisms. Three results exceeded the *Imperative* standard concentration of 2,000 cfu/100 ml specified for faecal coliforms. These were from: St Ouen Le Braye (19/06/2007), Victoria Pool (09/07/2007) and La Haule (24/09/2007). The sample from St Ouen Le Braye also had a total coliform concentration in excess 10,000 cfu/100ml. Both concentrations in this sample exceed the upper limit of detection for the coliform parameters (20,000 cfu/100 ml). This result was extremely unusual for this location, which demonstrated low coliform concentrations in all other samples through the 2007 bathing season and has consistently demonstrated low concentrations during past bathing seasons. In addition, the sample from 19/06/2007 exhibited a low faecal streptococci concentration (7 cfu/100 ml) whilst low concentrations were recorded for all three parameters at the St Ouen Watersplash location on the same day (<100 total coliforms/100 ml, 10 faecal coliforms/100 ml and 3 faecal streptococci/100 ml).

Weather conditions were fine on the sampling day in question. An additional sample taken as soon as possible after receiving the result (i.e. on 21/06/2007) demonstrated low concentrations for the two coliform parameters, below the lower limits of detection. These results suggest that the high total and faecal coliform concentrations from 19/06/2007 may not have resulted from faecal contamination (i.e. a corresponding elevated faecal streptococci concentration would have been expected in the sample). This could have been assessed by further microbiological analysis, for example confirmation of Escherichia coli from the faecal coliform plates. Such confirmatory analysis would provide some clue as to whether the organisms have a faecal provenance. Noncompliance with the *Guideline* criterion for total coliforms (i.e. 80% samples _ 500 cfu/100 ml) was restricted to La Haule, in the west of St Aubin's Bay on the south coast (Figure 1.4). This location also showed non-compliance with the *Guideline* criteria for the other two parameters (Table 1.2). In addition to La Haule, Guideline compliance failures associated with faecal coliforms were found at four further locations: Victoria Pool in the east of St Aubin's Bay, Grouville on the east coast, Bonne Nuit on the north coast and Rozel in the north east (Table 1.2, Figure 1.5). These locations were also non-compliant with the *Guide* standard for faecal streptococci (Table 1.2, Figure 1.6). Guideline non-compliance with the faecal streptococci parameter was evident at three further locations: Havre des Pas and Green Island on the south east coast and Plemont in the north west of Jersey. Overall compliance with the *Guide* standards for the 2007 season was thus 7/16 (43.8%), which is the lowest level recorded in Jersey in recent years (68.8% to 87.5% between 2003 and

2006). Comparable figures for England and Wales ranged from (80.4% to 85.2% between 2003 and 2006 – see: www.environment-agency.gov.uk) and the *Guideline* compliance

rate for 2007 was 79.8%.

3 Table 1.2 indicates that non-compliance with the faecal streptococci *Guide* criterion was the driver of the low overall level compliance with the *Guide* standards, with four of the nine non-compliant locations showing non-compliance with the faecal streptococci standard alone. Closer inspection of individual results with faecal streptococci concentrations exceeding 100 cfu/100 ml show some unusual patterns, with faecal streptococci concentrations exceeding the corresponding coliform parameter concentrations. Examples include La Haule, Victoria Pool, Grouville, Archirondel, Bouley Bay and Rozel on 14/05/2007 (Table 1.1, Figures 1.2 and 1.3). This pattern is also evident in samples from various locations on 15/05/2007 and is clearly implicated in the Guide compliance failures at Plemont and Green Island. Whilst it is unusual for faecal streptococci concentrations to exceed those of the coliform parameters such patterns have been observed elsewhere (Wyer et al., 1999). As with the sample exhibiting unusually high total and faecal coliform concentrations from St Ouen Le Braye discussed above, these unusual results could be explored through further microbiological analysis, for example the confirmation of intestinal enterococci. This would provide a more definitive answer as to whether the organisms originate from faecal contamination and are not environmental species of bacteria (e.g. aerobic spore bearing bacilli and Aerococcus viridans) that can contribute to presumptive faecal streptococci counts.

It is apparent from the decline in the quality of bathing water and the changes in the classification of designated shellfish harvesting areas that there is an environmental problem. This problem needs remediation before it is too late for many shellfish populations, and in turn the livelihoods of fishermen and public health. There is a steady decline so we must look at what is potentially the most environmentally hazardous source, and this is the reclamation site.

Heavy metals:

Heavy metals are individual metals and metal compounds that can impact human health. These are all naturally occurring substances which are often present in the environment at low levels. In larger amounts, they can be dangerous. Generally, humans are exposed to these metals by ingestion (drinking or eating) or inhalation (breathing). Working in or living near an industrial site which utilizes these metals and their compounds increases ones risk of exposure, as does living near a site where these metals have been improperly disposed. Subsistence lifestyles can also impose higher risks of exposure and health impacts because of hunting and gathering activities, such as fishing.

The most common are mercury, lead, cadmium, zinc, chromium and plutonium. Ecosystems can be affected by their discharge, e.g. marine and freshwater diatoms can lose 50 per cent of their growth rate at concentrations as low as 1 part per thousand million of mercuric compounds.

An important note on the leaching of heavy metals is the variation of each metal in terms of solubility. For example, if you took five samples with identical soil and water

quantities but tested these samples at different times (say 5 hour intervals), you may have substantially different results. Therefore the samples from the trench may not be a true representation of the total quantities of heavy metals that are available to the water stream.

There are numerous, complex effects of heavy metals and varying reactions, which for the purpose of this report may be too in depth. However I would like to highlight the fact that copper can be highly toxic to some shellfish in small concentrations. Also there is a link between the increased absorption of some heavy metals and certain strains of e-coli, this is because the heavy metals are also absorbed by the e-coli bacteria. For further information on this please read Appendix 3.

In a paper written in respect of the then proposed dumping of ash in the south of La Collette site, Mr. Tony Legg considered the potential for Cadmium to leach –

"From the above observations it is evident that an oxidising environment that is high in chlorine, that fluctuates in salinity, that occasionally is anoxic with free sulphide ions, that has a water table comprised of tidal and field water capacity interstitial solute, and has energy put into the system by tidal movement of that solute, is very well suited to making cadmium bioavailable. In addition, the ash material is already in an oxidised state, thereby speeding up the process.

"In situ, the ash is likely to be just above the MHWS mark and some 2 meters deep with c. 1 meter overburden. After normal rainfall or windblown spray this zone will be at field capacity with water filling all available pore spaces. When the tide rises or falls this water will be moved and exchanged, salinities will change, waters will drain and cadmium enriched leachate will form."

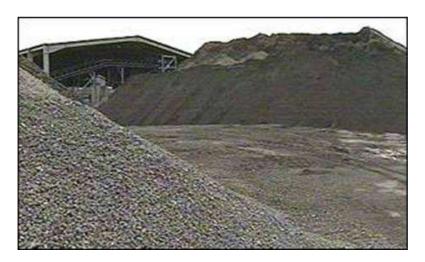
Information on heavy metals from the States of Jersey Website:

Accessed 03/04/09, States of Jersey website, information on heavy metals: http://www.gov.je/Health/public_health/health_protection/pollution/Heavy+Metals.htm

Heavy Metals

The sampling for Heavy Metals is now carried out by the Environment Department.

In many industrialised countries, including Jersey, incineration of refuse is considered a convenient method of reducing waste volume since it commonly achieves a reduction of about 90%. However, in the broader environmental context, the process is not without disadvantages particularly with respect to the associated gaseous emissions.



The solid residue consists primarily of bottom ash (80 - 90%) from the furnace bottom and fly ash from the dust collection system. (see picture above) Whilst in some instances incineration residues may be treated as toxic waste (eg Canada), it is not uncommon for them to be disposed of by landfilling. However, in recent years there has been an increased concern for the potential hazards posed by leachate from such sites (eg Hjelman et al, 1988). Leachate would contain heavy metals such as cadmium, mercury and lead which can be toxic at relatively low concentrations.

The lack of suitable inland disposal sites has meant that Jersey has increasingly turned to reclamation as the sole means of disposing of solid waste, with incineration playing an important role in reducing the volume of waste and prolonging the life of reclamation sites. However the possibility of leachate entering the marine environment is very real. If this occurred locally it would constitute a breach of the Dumping at Sea Law Sea Fisheries (Miscellaneous Provisions) 1974. This has led to the ash being dumped since 1987 above mean high water level at the reclamation sites.

When such leaching is excessive and/or prolonged, the metals can become concentrated up a marine food chain. Some bivalves, for example, concentrate certain metals tens of thousand times above the ambient level (Brooks and Rumsby, 1965). In extreme cases, potential public health risks arise because of the ingestion of contaminated seafood.

In Jersey the common limpet and brown fucoid seaweed have been sampled since 1994 at five locations (ie West of Albert, La Collette, St Aubin, Corbiere and Gorey). Samples have also been taken from Havre des Pas and Les Ecrehous.

Please contact the Environment Department on Tel: 441600 for more information.

Bioaccumulation

Biological concentration is the mechanism whereby filter feeders such as limpets, oysters and other shellfish concentrate heavy metals or other stable compounds present in dilute concentrations in sea or fresh water. Animals can accumulate metals as well by eating plants, fish, or drinking water with elevated metal concentrations. These metals are not excreted by the animals; rather, they accumulate mostly in the organs as well as the skin, hair, and bones. Fish accumulate metals from the water they live in as well as from organisms they eat. Bottom feeders are particularly susceptible to metals bioaccumulation as they can ingest sediments laced with metals. Minute traces of toxic compounds can be concentrated biologically and enter the food chain of human beings.

Potential effects on human health of some heavy metals:

Mercury

Mercury is the only metal which is liquid at ordinary temperatures. It rarely occurs free in nature and is a heavy, silvery-white liquid metal.

Once consumed, mercury and the bivalent metals are engaged in a continuous fight against one another which results in the replacement of the "lighter" element by the "heavier" one, in terms of their atomic masses. Replacement reactions, also called "fight for the site," occur when heavy metals grab the biological spaces that should be filled by necessary organic minerals. Just as carbon monoxide replaces essential oxygen, other elements and compounds cause their toxic effect by replacing chemicals essential to biochemical functions. Mercury, found in amalgam fillings, paints, and some industrial processes, is not recognized in having any use in the body. Mercury is not taken up by plants, however, it may turn up in food as it can be spread within food chains by smaller organisms which are consumed by humans, and one example is through fish. Concentrations of Mercury in fish usually greatly exceed the concentrations in their environment. Beef products can also contain eminent quantities of mercury. Mercury is not commonly found in plant products, but it can enter our bodies through vegetables and other crops, when sprays containing mercury are applied in agriculture.

Adverse Health effects of Mercury:

Mercury salts will compete with zinc in its bio-chemical reactions hence preventing zinc performing its functions in the body. Therefore the leaching of mercury into the body from whatever source will cause zinc deficient symptoms to appear such as fatigue, PMS, thyroid problem, loss of smell and taste, macular degeneration, prostate enlargement, rheumatoid arthritis, sterility, immune suppression, etc., even if there is plenty of zinc available. Studies show that mercury is eight times more concentrated in the foetus than in the rest of the body. Direct exposure to mercury can cause lung

irritation, skin rashes, nerve, brain and kidney damage, eye irritation, vomiting and diarrhoea. Mercury and its many effects on our bodies at elevated levels can be simplified into the following main effects:

- Disrupting the nervous pathways;
- Damage to brain function, can cause degradation of learning abilities, personality changes, tremors, vision changes, deafness, and muscle in-coordination and memory loss;
- DNA damage and chromosomal damage chromosomal damage is known to cause mongolism;
- Allergic reactions, resulting in skin rashes, tiredness and headaches;
- Negative reproductive effects, such as sperm damage, birth defects and miscarriages.

Regulatory limits:

- EPA 2 parts per billion parts (ppb) in drinking water
- FDA 1 part of methylmercury in a million parts of seafood.
- \bullet OSHA -0.1 milligram of organic mercury per cubic meter of workplace air and 0.05 milligrams per cubic meter of metallic mercury vapor for 8-hour shifts and 40-hour work week.

Cadmium

Cadmium is a very toxic metal. All soils and rocks, including coal and mineral fertilizers, contain some cadmium. Cadmium has many uses, including batteries, pigments, metal coatings, and plastics. It is used extensively in electroplating.

Cadmium, in industry is a by-product from the extraction of zinc, lead and copper. Cadmium is found in pesticides and manures therefore are seen to enter the environment from terra-forming. People's uptake of cadmium takes place mainly through food. Fish, plants and animals bio-accumulate. Examples: liver, kidney, meat, mushrooms, shellfish, mussels, cocoa powder and dried seaweed. An exposure to significantly higher cadmium levels occur when people use tobacco. The cadmium in tobacco smoke enters the bloodstream via the respiratory system and distributed to the rest of the body. Cadmium can severely damage the lungs and may even cause death.

Once the cadmium reaches the liver where it is bonded to protein forming complexes, which are then transported to the kidneys where accumulation causes damage to the filtration process. This damage allows essential proteins and glycol nutrients to be excreted from the body causing even further kidney damage.

Health effects that can be caused by cadmium are:

- Damage to the central nervous system
 Damage to the immune system
 Psychological disorders and possibly DNA damage
- Reproductive failure and possibly even infertility;

- Cadmium and cadmium compounds are known human carcinogens. Smokers get exposed to significantly higher cadmium levels than non-smokers. Severe damage to the lungs may occur through breathing high levels of cadmium.
- Ingesting very high levels severely irritates the stomach, leading to vomiting and diarrhea.
- Long-term exposure to lower levels leads to a buildup in the kidneys and possible kidney disease, lung damage, and fragile bones.

Regulatory limits

- \bullet EPA 5 parts per billion (ppb) or 0.005 parts per million (ppm) of cadmium in drinking water
- Food and Drug Administration (FDA) concentration in bottled drinking water should not exceed 0.005 ppm (5 ppb).
- OSHA an average of 5 micrograms per cubic meter of workplace air for an 8-hour workday, 40-hour work week.

Lead

As a result of human activities, such as fossil fuel burning, mining, and manufacturing, lead and lead compounds can be found in all parts of our environment. This includes air, soil, and water. Lead is used in many different ways. It is used to produce batteries, ammunition, metal products like solder and pipes, and X-ray shielding devices. Lead is a highly toxic metal and, as a result of related health concerns (see below), its use in several products like gasoline, paints, and pipe solder, has been drastically reduced in recent years. Today, the most common sources of lead exposure are lead-based paint and possibly water pipes in older homes, contaminated soil, household dust, drinking water, lead crystal, lead in certain cosmetics and toys, and lead-glazed pottery. Lead can be present in drinking water as a result of dissolution from natural sources, or from household plumbing systems containing lead. These may include lead in pipes, or in solder used to seal joins. The amount of lead dissolved will depend on a number of factors including pH, water hardness, and the standing time of the water. Lead is the most common of the heavy metals and is mined widely throughout the world. It is used in the production of lead acid batteries, solder, alloys, cable sheathing, paint pigments, rust inhibitors, ammunition, glazes and plastic stabilisers. The organo-lead compounds tetramethyl and tetraethyl lead are used extensively as anti-knock and lubrication compounds in gasoline.

Adverse Health effects of Lead

Lead fulfils no essential function in the human body and is a probable human carcinogen. Lead can affect every organ and system in the body. Long-term exposure of adults can result in decreased performance in some tests that measure functions of the nervous system; weakness in fingers, wrists, or ankles; small increases in blood pressure; and anemia. Exposure to high lead levels can severely damage the brain and kidneys and

ultimately cause death. In pregnant women, high levels of exposure to lead may cause miscarriage. High level exposure in men can damage the organs responsible for sperm production.

Also:

- Behavioural disruptions of children, such as aggression, impulsive behaviour and hyperactivity.
- Disruption of the biosynthesis of haemoglobin and anaemia;
- Declined fertility of men through sperm damage;
- Diminished learning abilities of children;
- Loss of I.Q;
- Kidney damage;
- Rise in blood pressure;
- Disruption of nervous systems;

Regulatory limits:

● EPA – 15 parts per billion (ppb) in drinking water, 0.15 micrograms per cubic meter in air.

Copper

Copper is widely distributed in rocks and soils as carbonate and sulphide minerals. Copper is relatively resistant to corrosion and is used in domestic water supply pipes and fittings. It is also used in the electro-plating and chemical industries, and in many household goods. Copper sulphate is used extensively to control the growth of algae in water storages. Copper can be found in many kinds of food and in drinking water, because of that, we absorb eminent quantities of copper each day by eating, drinking and breathing. Organic copper is necessary as a trace element that is essential for human health.

Long-term exposure to copper in the industry level can cause irritation of the nose, mouth and eyes and it causes headaches, stomach-aches, dizziness, vomiting and diarrhoea. Intentionally high uptakes of copper may cause liver and kidney damage and even death.

Some adverse health effects of copper:

- Insomnia:
- Depression;
- Hypo-tension;
- Acne:
- Heart disease:
- Pre-menstrual tension;
- Postpartum depression;
- Paranoid and hallucinatory schizophrenias

- Childhood hyperactivity and autism.
- Above 50mg/Kg body weight can be lethal.

Zinc

Zinc is a very common substance and many foodstuffs contain certain concentrations of zinc. Drinking water also contains certain amounts of zinc, which may be higher when it is stored in metal tanks. Organic zinc is a trace element that is essential for human health. People deficient in zinc absorption can experience a loss of appetite, decreased sense of taste and smell, slow wound healing, skin sores and even birth defects.

Adverse Health effects of Zinc

Some adverse health effects of Zinc when over exposed:

- Stomach cramps;
- Skin irritations:
- Vomiting, nausea, anaemia;
- Arteriosclerosis;
- Respiratory disorders.

Arsenic

Aside from occurring naturally in the environment, arsenic can be released in larger quantities through volcanic activity, erosion of rocks, forest fires, and human activity. Arsenic is also found in paints, dyes, metals, drugs, soaps and semi-conductors. Animal feeding operations and certain fertilizers and pesticides can release high amounts of arsenic to the environment as can industry practices such as copper or lead smelting, mining, and coal burning.

Some health effects:

- Arsenic is odorless and tasteless. Inorganic arsenic is a known carcinogen and can cause cancer of the skin, lungs, liver and bladder.
- Lower level exposure can cause nausea and vomiting, decreased production of red and white blood cells, abnormal heart rhythm, damage to blood vessels, and a sensation of "pins and needles" in hands and feet.
- Ingestion of very high levels can possibly result in death.
- Long-term low level exposure can cause a darkening of the skin and the appearance of small "corns" or "warts" on the palms, soles, and torso.

Fish and shellfish can accumulate arsenic and is also toxic to plants at levels not harmful to humans and animals.

Regulatory limits:

- Environmental Protection Agency (EPA) 0.01 parts per million (ppm) in drinking water.
- Occupational Safety and Health Administration (OSHA) 10 micrograms per cubic meter of workplace air ($10 \mu g/m_3$) for 8 hour shifts and 40 hour work weeks.

Barium

Barium is a very abundant, naturally occurring metal and is used for a variety of industrial purposes. Barium compounds, such as barium-nickel alloys are used for spark-plug electrodes and in vacuum tubes as a drying and oxygen-removing agent; barium sulfide is used in fluorescent lamps; barium sulfate is used in diagnostic medicine; barium nitrate and chlorate give fireworks a green color. Barium compounds are also used in drilling muds, paint, bricks, ceramics, glass, and rubber. Barium accumulates in certain plants, seaweed, and fish.

Health effects

Barium is not known to cause cancer.

- Short term exposure can cause vomiting, abdominal cramps, diarrohea, difficulties in breathing, increased or decreased blood pressure, numbness around the face, and muscle weakness.
- Large amounts of barium intake can cause, high blood pressure, changes in heart rhythm or paralysis and possibly death.

Regulatory limits:

- EPA 2.0 parts per million (ppm) in drinking water.
- OSHA 0.5 milligrams of soluble barium compounds per cubic meter of workplace air for 8 hour shifts and 40 hour work week.

Chromium

Chromium is found in rocks, animals, plants, and soil and can be a liquid, solid, or gas. Chromium compounds bind to soil and are not likely to migrate to ground water but, they are very persistent in sediments in water. Chromium is used in metal alloys such as stainless steel; protective coatings on metal (electroplating); magnetic tapes; and pigments for paints, cement, paper, rubber, composition floor covering and other materials. Its soluble forms are used in wood preservatives. Chromium has a high potential for uptake by aquatic life, especially bottom-feeders.

Health effects:

Chromium (VI) compounds are toxins and known human carcinogens, whereas Chromium (III) is an essential nutrient. Breathing high levels can cause irritation to the lining of the nose; nose ulcers; runny nose; and breathing problems, such as asthma, cough, shortness of breath, or wheezing. Skin contact can cause skin ulcers. Allergic reactions consisting of severe redness and swelling of the skin have been noted. Long term exposure can cause damage to liver, kidney circulatory and nerve tissues, as well as skin irritation.

Regulatory limits

- EPA- 0.1 ppm (parts per million) in drinking water.
- FDA should not exceed 1 milligram per liter (1ppm) in bottled water.
- OSHA an average of between 0.0005 and 1.0 milligram per cubic meter of workplace air for an 8-hour workday, 40-hour workweek, depending on the compound.

Selenium

Selenium is a trace mineral widely distributed in most rocks and soils. Processed selenium is used in the electronics industry; as a nutritional supplement; in the glass industry; in plastics, paints, enamels, inks, and rubber; in the preparation of pharmaceuticals; as a nutritional feed additive for poultry and livestock; in pesticide formulations; in rubber production; as an ingredient in antidandruff shampoos; and as a constituent of fungicides. Radioactive selenium is used in diagnostic medicine. Selenium Biocaccumulates readily; Indicator plants for high selenium concentrations in soils are: milkvetch or locoweed (Astragalus), prince's plume (Stanleya), woody aster (Xylorhiza, sp.) and false goldenweed (Oonopsis, sp.) These plants require high selenium levels to thrive. High concentrations are found in kidney, tuna, crab and lobster.

Health effects:

Selenium is toxic in large amounts, but trace amounts of it are necessary for cellular function in most, if not all, animals. For humans, selenium is an essential trace nutrient. For example, selenium plays a role in the element functioning of the thyroid gland. The Tolerable Upper Intake Level is 400 micrograms of selenium per day. Consumption above that level can lead to selenosis. Short-term oral exposure to high concentrations can cause nausea, vomiting, and diarrhea. Chronic oral exposure to high concentrations can produce selenosis. Major signs of selenosis are hair loss, nail brittleness, and neurological abnormalities. Brief exposures to high levels in air can result in respiratory tract irritation, bronchitis, difficulty breathing, and stomach pains. Longer-term exposure can cause respiratory irritation, bronchial spasms, and coughing.

Regulatory limits

- EPA 50 parts per billion of selenium (50 ppb) in drinking water.
- OSHA 0.2 mg per cubic meter of workroom air for an 8-hour work shift.

Silver

Silver usually combines with other elements such as sulfide, chloride, and nitrate. Silver is used to make jewelery, silverware, electronic equipment, and dental fillings. Silver metal is also used in electrical contacts and conductors, in brazing alloys and solders, and in mirrors. Silver compounds are used in photographic film. Dilute solutions of silver nitrate and other silver compounds are used as disinfectants and as an antibacterial agent. Silver iodide has been used in attempts to seed clouds to produce rain.

Health effects:

Exposure to high levels for a long period may result in a condition called arygria, a blue-gray discoloration of the skin and other body tissues. Argyria appears to be a cosmetic problem that may not be otherwise harmful to health. Exposure to high levels of silver in the air has resulted in breathing problems, lung and throat irritation, and stomach pains. Skin contact with silver can cause mild allergic reactions such as rash, swelling, and inflammation in some people.

Regulatory limits:

- EPA recommends concentration in drinking water not to exceed 0.10 parts per billion (ppb). Requires that spills or accidental releases of 1,000 pounds or more be reported.
- OSHA in workplace air, 0.01 milligrams per cubic meter (0.01 mg/m³) for an 8-hour workday, 40-hour workweek.

Concerns about heavy metals:

The Dangerous Substances Directive of the European Union (76/464/EEC) defines dangerous chemicals as those which are toxic, persistent and / or bioaccumulative. As they are elements, they cannot be broken down; therefore heavy metals will persist in the environment. Unlike many organic pollutants, which eventually degrade to carbon dioxide and water, heavy metals will tend to accumulate in the environment, especially in lake, estuarine or marine sediments. Metals can be transported from one environment compartment to another.

Whether the source of heavy metals is natural or anthropogenic, the concentrations in terrestrial and aquatic organisms are determined by the size of the source and adsorption/precipitation in soils and sediments. The extent of adsorption depends on the metal, the absorbent, the physio-chemical characteristics of the environment (e.g. pH, water hardness and redox potential) and the concentrations of other metals and complex chemicals present.

Critical levels and loads:

Most critical levels derive from the European Union's Dangerous Substances Directive (76/464/EEC) and the US EPA (US EPA 1998) for water and the EU Air Quality Framework Directive (96/62/EEC) and the World Health Organization (World Health

Organization 1987) for air. The first Air Quality Daughter Directive sets critical levels for lead and another in preparation will sets limits for cadmium, arsenic, nickel and mercury. The general aim of the Air Framework and Daughter Directives is to avoid, prevent and reduce harmful effects of air pollutants on human health and the environmental as a whole. The National Air Quality Strategy is the means by which this European Union legislation is being implemented in the United Kingdom. The EU sludge to land directive (86/278/EEC) sets critical levels for heavy metals in agricultural soils that receive sewage sludge.

The UK is obliged under the Dangerous Substances Directive to set Environmental Quality Standards (EQSs; critical levels) for selected (List I and II) chemicals in water which are of national concern and the Environmental Protection Act 1990 requires EQSs for further chemicals (the Red List) in order to protect the aquatic environment. Critical levels for mercury and cadmium are available and one for arsenic is imminent.

Conclusions:

The EEC Directives clearly state the requirements for the quality of shellfish, and heavy metals are mentioned in this 1979 Directive. (See Appendix 4).

It states, inter alia, that the concentration of each heavy metal in shellfish flesh must be so limited that it contributes in accordance with Article 1, to the high quality of shellfish products

Article 1:

This Directive concerns the quality of shellfish waters and applies to those coastal and brackish waters designated by the Member States as needing protection or improvement in order to support shellfish (bivalve and gasteropod molluscs) life and growth and thus to contribute to the high quality of shellfish products directly edible by man.

In able to ascertain a greater understanding and put together a comprehensive analysis of the situation, we would need to be able to access various data from States Departments to compile background information. There appears to be difficulties in obtaining information from the various departments and there is only limited data which is available to the public for viewing.

A full and comprehensive risk analysis needs to be undertaken as soon as possible to limit any further damage to the marine environment and in turn public health.

A comprehensive monitoring program needs to be independently established as soon as possible. Save Our Shoreline does not own the necessary monitoring equipment to be able to do this, however all results could be interpreted and collated to provide a full analysis of the situation.

Example of factors to be taken into account during monitoring:

- Scope of monitoring sediment, soil, leachate, biological indicators, sea water samples (ideally from St Aubins, Elizabeth Castle to Gorey at specified intervals), testing of various shellfish/ fish/ lobsters for heavy metals which are for sale to the public, aquatic plants etc.
- Preparation of list of key pollutants to test for
- Tidal flows, weather, season, sewage outflows, other pollution incidents etc.

Perhaps one of the most important aspects to analyse is the sediment. The sediment is the deposit of silt and accumulated organic and /or inorganic materials at the bottom of rivers, lakes, seas, etc. sediments can act as sinks for pesticides and heavy metals and can contain concentrations as much as 800 times that of water in the case of dieldrin (one of the group of chlorinated hydrocarbons). Chlorinated hydrocarbons are one of the three major groups of synthetic insecticides. This group includes endosulfan which is highly toxic to fish. In general, in fishes they have the effect of preventing oxygen uptake, causing suffocation. These hydrocarbons are part of the organochlorines group, which are characterized by their persistence, mobility and high biological activity. They have very long half lives and have a high capacity to injure living systems and allied with their other attributes may possibly constitute the greatest threat to our life-supporting ecosystems and associated biological cycles. The deposits of sediment are therefore a source of secondary contamination as well as allowing the bottom feeders to accumulate the pollutants at much greater concentrations than water analysis would indicate, thereby contaminating the food chain. As we have strong undercurrents in places it would be entirely feasible that sections of highly polluted sediment could be moved around the bay having almost a tidal effect on the sea-bed, therefore circulating the 'problem' around.

The problem is complicated and there are no easy answers, but it is possible to control NPS pollution and nature will respond to restoration efforts. The best way to solve our NPS pollution problems is through effective local government and individual citizen intervention.

An example of pollution potential:



One gallon of used motor oil can pollute up to 2 million gallons of water.

End note:

Not all possible pollutants to the marine environment have been covered in this report.

APPENDIX 1

The relationship between the standard of the EIS and the Planning and Building (Jersey) Law 2002

Note: (In English law it is referred to as an Environmental Impact Assessment with the non-technical summary known as the Environmental Impact Statement – in can be found in the Building Regulations 2005)

In Jersey law it is referred to as an Environmental Impact Statement with the non-technical summary known as the Environmental Statement.)

The EIS comes under Article 13 of the Planning and Building (Jersey) Law.

The Planning and Building (Environmental Impact) (Jersey) Order 2006, is in pursuance of Article 13 of the Planning and Building (Jersey) Law 2002.

In other words this Order is an extension of Article 13.

The Planning and Building (Environmental Impact) (Jersey) Order 2006

The incinerator comes under the section 11(4) Schedule 1, that requires an EIS by law and has to meet the criteria of Schedule 2.

(1) Except as provided by paragraph (2), proposed development specified in column 1 of Schedule 1 is prescribed development for the purpose of Article 13(1)(a) of the Law.

SCHEDULE 1

DESCRIPTIONS OF DEVELOPMENT IN RESPECT OF WHICH AN ENVIRONMENTAL IMPACT STATEMENT IS REQUIRED

11 Other projects

(4) The construction of an installation for the disposal or treatment of waste (including waste from mines and quarries and vegetable waste).

Therefore all aspects of the EIS have to be met by Law.

WHAT AN ENVIRONMENTAL IMPACT STATEMENT MUST CONTAIN

PART 1

- 3. A description of the aspects of the environment likely to be significantly affected by the development, including, in particular, population, fauna, flora, soil, water, air, climatic factors, material assets, including the architectural and archaeological heritage, landscape and the inter-relationship between the above factors.
- 4. A description of the likely significant effects of the development on the environment, which should cover the direct effects and any indirect, secondary, cumulative, short, medium and long-term, permanent and temporary, positive and negative effects of the development, resulting from –
- (a) the existence of the development;
- (b) the use of natural resources;

(c) the emission of pollutants, the creation of nuisances and the elimination of waste,

and the description by the applicant of the forecasting methods used to assess the effects on the environment.

5. A description of the measures envisaged to prevent, reduce and where possible offset any significant adverse effects on the environment.

There has been a failure, by omission in Articles 3, 4 and 5 where no inter-relationship has been assessed between factors. Ramsar – Air pollutants, also leaching through ground soil to sea – from waste storage etc. As a result of the absence of this information, no mitigation has been put into place for environmental and human health in the EIS.

Under Article 13 (3) of the Planning and Building (Jersey) Law 2002, the Minister has to take the statement into account in determining the application.

13 Environmental impact of proposed development

(3) The Minister shall take the statement into account in determining the application.

Please note the part about a representation with a material omission below, with reference to the omission above under Articles 3, 4 and 5.

PART 3

PLANNING CONTROL

10 False information, etc. in application for planning permission

(1) If when making an application for planning permission a person knowingly or recklessly makes a false or misleading statement or representation or a statement or representation with a material omission

The following remedial action can be taken by the Minister for a representation with material omission – revoking the planning permission if it has already been granted.

- (2) If a person has made such a statement or representation and the planning permission has been granted, the Committee may –
- (a) revoke or modify the permission

Relevant parts of the Laws:

Planning and Building (Jersey) Law 2002 PART 3

PLANNING CONTROL

10 False information, etc. in application for planning permission

- (1) If when making an application for planning permission a person knowingly or recklessly makes a false or misleading statement or representation or a statement or representation with a material omission the person shall be guilty of an offence and liable to imprisonment for a term of 2 years and a fine.[7]
- (2) If a person has made such a statement or representation and the planning permission has been granted, the Committee may –

- (a) revoke or modify the permission; and
- (b) if the development has been started or undertaken, serve a notice on the owner of the land to which the permission relates.
- (3) The notice may require the owner of the land, within a period specified in the notice –
- (a) to undertake work specified in the notice to restore the land to its condition before the development was undertaken; or
- (b) to modify the development to the extent specified in the notice.
- (4) The work to be undertaken may include –
- (a) the demolition or alteration of the whole or any part of a building; or
- (b) the discontinuance of a use of land.
- (5) The Minister may act in accordance with paragraph (2) whether or not proceedings have been taken in respect of the offence under paragraph (1).
- (6) A person who –
- (a) fails to comply with a notice served on the person in accordance with paragraph (2)(b); or
- (b) uses land in contravention of the notice,

shall be guilty of an offence and liable to a fine of level 3 on the standard scale.

- (7) If at the end of the period for compliance specified in a notice under paragraph (2)(b), work required by the notice to be undertaken has not been undertaken, the Minister may enter the land and undertake the work.
- (8) The expenses reasonably incurred by the Minister in undertaking work in accordance with paragraph (7) shall be recoverable as a debt due to the Minister from the person in default.
- (9) The Minister may undertake work in accordance with paragraph (7) whether or not proceedings have been taken under paragraph (6).

(10) Action taken by the Minister under this Article does not give any person the right to claim compensation in respect of any loss or damage the person may suffer as a result of the action.

13 Environmental impact of proposed development

- (1) This Article applies in respect of an application for planning permission –
- (a) to carry out development that falls within a class of development prescribed for the purpose of this sub-paragraph; or
- (b) where the Minister is satisfied that if the proposed development were to be carried out it would be likely to have a significant effect on the environment of Jersey or elsewhere.
- (2) Where this Article applies the Minister shall not consider the application until the applicant has provided the Minister with an environmental impact statement.
- (3) The Minister shall take the statement into account in determining the application.
- (4) The Minister shall by Order prescribe for the purpose of paragraph (1)(a) classes of development in respect of which an environmental impact statement is required.

- (5) The Order shall also prescribe –
- (a) the particulars an environmental impact statement must contain;
- (b) the qualifications of the people by whom those particulars are to be provided;
- (c) the form an environmental impact statement is to take; and
- (d) such other matters as the Minister considers relevant to the preparation and provision of an environmental impact statement.

16 Development of concern to the Minister for Transport and Technical Services

- (1) This Article applies in respect of an application for planning permission for development that falls within an area of responsibility or concern of the Minister for Transport and Technical Services.
- (2) Where this Article applies the Minister shall refer the application to the Minister for Transport and Technical Services for comment.
- (3) The Minister shall in determining the application take into account any comment made by the Minister for Transport and Technical Services in respect of the matters specified in paragraph (4).
- (4) Those matters are –
- (a) the sufficiency of any sewerage or drainage system, flood defence work or water course that may be affected by the development, the prevention of damage to it, and any hindrance to its repair or maintenance;
- (b) the limitation of damage by surface water that could be caused by the development;

(c) the effect of the development on water quality (including sea water quality).

Planning and Building (Environmental Impact) (Jersey) Order 2006

2 Prescribed development requiring an environment impact statement

- (1) Except as provided by paragraph (2), proposed development specified in column 1 of Schedule 1 is prescribed development for the purpose of Article 13(1)(a) of the Law.
- (2) If a qualifying criterion is specified in column 2 in respect of any proposed development, it is not prescribed development for the purpose of Article 13(1)(a) of the Law unless it meets that criterion or, if more than one criterion is specified in column 2, at least one such criterion.
- (3) Where proposed development mentioned in paragraph (1) is already authorised, executed or in the process of being executed, a change or extension of the development is also prescribed development for the purpose of Article 13(1)(a) of the Law.
- (4) Development mentioned in paragraph (1) or paragraph (3) is not prescribed development for the purpose of Article 13(1)(a) of the Law if the Minister is satisfied that by virtue of factors such as the nature, size or location

of the proposed development it would be unlikely, if carried out, to have	a
significant effect on the environment, either of Jersey or elsewhere.	

SCHEDULE 1

DESCRIPTIONS OF DEVELOPMENT IN RESPECT OF WHICH AN ENVIRONMENTAL IMPACT STATEMENT IS REQUIRED

11 Other projects

(4) The construction of an installation for the disposal or treatment of waste (including waste from mines and quarries and vegetable waste).

The change of use of land to use as a site for the storage of scrap metal. The land is within 100 metres of a stream, pond or reservoir.

SCHEDULE 2

(Article 1)

WHAT AN ENVIRONMENTAL IMPACT STATEMENT MUST CONTAIN

PART 1

- 1. Description of the development, including in particular –
- (a) a description of the physical characteristics of the whole development and the land-use requirements during the construction and operational phases;
- (b) a description of the main characteristics of the production processes, for instance, nature and quantity of the materials used;
- (c) an estimate, by type and quantity, of expected residues and emissions (water, air and soil pollution, noise, vibration, light, heat, radiation, etc.) resulting from the operation of the proposed development.
- 2. An outline of the main alternatives studied by the applicant and an indication of the main reasons for his or her choice, taking into account the environmental effects.
- 3. A description of the aspects of the environment likely to be significantly affected by the development, including, in particular, population, fauna, flora, soil, water, air, climatic factors, material assets, including the architectural and archaeological heritage, landscape and the inter-relationship between the above factors.
- 4. A description of the likely significant effects of the development on the environment, which should cover the direct effects and any indirect, secondary, cumulative, short, medium and long-term, permanent and temporary, positive and negative effects of the development, resulting from –
- (a) the existence of the development;

- (b) the use of natural resources;
- (c) the emission of pollutants, the creation of nuisances and the elimination of waste,

and the description by the applicant of the forecasting methods used to assess the effects on the environment.

- 5. A description of the measures envisaged to prevent, reduce and where possible offset any significant adverse effects on the environment.
- 6. A non-technical summary of the information provided under paragraphs 1 to 5 of this Part.
- 7. An indication of any difficulties (technical deficiencies or lack of know-how) encountered by the applicant in compiling the required information.

PART 2

- 1. A description of the development comprising information on the site, design and size of the development.
- 2. A description of the measures envisaged in order to avoid, reduce and, if possible, remedy significant adverse effects.
- 3. The data required to identify and assess the main effects which the development is likely to have on the environment.
- 4. An outline of the main alternatives studied by the applicant and an indication of the main reasons for his or her choice, taking into account the environmental effects.
- 5. A non-technical summary of the information provided under paragraphs 1 to 4 of this Part

APPENDIX 2

PUBLIC HEALTH SERVICES

Contaminated sites

RESPONSES TO Sen. Syvret's questions

(i) Was it accepted that the combined ash from the island's municipal waste incinerator contaminated many parts of the St. Helier waterfront site?

The West of Albert land reclamation operation was undertaken initially by the mixing of combined incinerator ash and inert waste at the point of disposal until 1987 when a legislative review prompted a change in disposal practise. Thereafter the incinerator ash was singularly deposited in a number of pits above mean high water mark at varying points across the reclamation site.

(ii) Was it accepted that the combined incinerator ash contained a variety of known human health hazards, including carcinogens and endocrine disrupters; for example cadmium, lead, mercury, zinc, copper Dioxins furans, PCBs etc?

Fly ash from Municipal Waste incineration contains heavy metals, dioxins, and furans. These products, which have varying levels of toxicity peculiar to the respective material, are proven to have an adverse affect on human health. Monitoring data for the Jersey incinerator, obtained in 1993 by Warren Spring Laboratory, confirms the presence of these materials both in fly ash and combined ash.

The main reference document for analysis of this ash, in the context of the disposal of ash at the West of Albert site, is a report compiled by consultants, WRc Alert in November 1995. This report refers to analysis undertaken in April 1995 and September 1995 at a number of locations including fresh material, stockpiled ash and material which had been in the ground for varying periods of time. The analysis identified a range of heavy metal contaminants including Arsenic, Mercury, Copper, Nickel, Zinc, Cadmium, Lead and Chromium. These types of contaminant fit the profile of expected contaminants arising from an incineration process².

It would appear that this report did not carry out an analysis of waste ash material for the presence of certain other contaminants, i.e. dioxins, although other analysis of ash material has been undertaken.

(iii) Was the ash a threat to the health of those who had been, or might have been exposed to it?

The toxic element associated with fly ash is mainly retained in the fine particulate fraction of the ash. There is a risk of ingestion, inhalation or absorption of this material into the human body if a person comes into direct contact with the ash, whether as a consequence of the handling, transport, deposit or excavation of the material, or the result of fugitive emissions from site.

¹ WRc Ref:CO 4028/1 Table 7, November 1995

² Table 3.4, Report 132, A guide for safe working on contaminated sites, Construction Industry Research and Information Association, 1996

(iv) If it was accepted that the ash was a human health hazard, was this new knowledge or had it been ascertainable from the beginning of the dumping?

The harmful health effects of heavy metals have been known about, and has been well documented for more than a century. The body of knowledge of dioxins, furans and PCBs is far more recent, from around 1980, although the ability to accurately measure and quantify their long term health effects is continuing to be explored. It is considered that at present there is no certain safe lower limit for exposure to these materials, which bio-accumulate in the environment and the body, but tolerable daily intake levels have been defined by the UK Department of Health and the World Health Organisation. The current quoted emission limits from combustion processes can be quantified as the lower limit of detection of these materials.

(v) Was it accepted that many - mainly construction - workers had been exposed to the ash with, until recently, no health and safety protection; for example, those who built the underpass?

It is apparent that the analysis which was carried out by WRc identified a range of toxic contaminants which are associated with potential ill health effects to persons exposed to the ash material³. However, it should be noted that, due to the nature of the waste management process, the amounts of contaminants in the material will vary, depending on the composition of the waste which was incinerated.

In considering the potential for occupational exposure, it is recognised that individual exposure may be through a number of paths including skin absorption, skin penetration, ingestion and inhalation. Landfill on the West of Albert site is believed to have commenced in 1986, with records of construction and civil engineering projects commencing in 1985 and continuing to the present day. There may therefore have been a potential for occupational exposure to the ash material at the West of Albert site since 1985 with the potential groups of workers at risk including those carrying out landfill operations, site investigation and ground works.

It is not however possible to state with any certainty whether or not such workers received significant occupational exposures for a number of reasons:

- As noted, the presence and level of contaminants will vary depending on the composition of the material.
- The site was also used for the deposit of inert materials with ash material forming only a part of the total material deposited. There was therefore not a continuous consistent potential for exposure,
- Weather conditions may have assisted in reducing the potential for exposure to dusts,
- Possible protective measures to prevent exposure to the ash material include the wearing of personal
 protective equipment and good hygiene facilities. Such measures may have been in place on the various
 projects carried out on the site,
- lack of air monitoring carried out prior to the Spie Batignolles Camerons development, which may have assisted in identifying the potential for airborne contaminated material, and
- lack of information about any individuals reporting ill health effects from working with the material.

³ Table 3.6, Report 132, A guide for safe working on contaminated sites, Construction Industry Research and Information Association, 1996

⁴ HS(G) 66, Protection of workers and the general public during the development of contaminated land, Health and Safety Executive, 1991.

It is concluded that the potential for occupational exposure to contaminated material was present on the West of Albert site, however, it has not been possible to determine whether individuals have suffered any ill health effects from this potential for exposure.

(vi) Was it accepted that dust contaminated by the ash could become airborne and thus form an inhalation and ingestion pathway to people both on the site and beyond its bounds?

During handling, transport, deposit and excavation the ash material has been found to be in an homogenous damp condition, and therefore the ability for fines from the material to be liberated and become airborne is limited. However, the subsequent piling operation at the Waterfront site saw the acrosol dispersion of liquefied fines material both on and off site and in to adjacent premises. The subsequent drying out of this material along with any material brought off site by vehicle tyres, chassis bodies and site operatives could result in fugitive emissions of fines when dry, with the potential to be carried by strong winds and passing traffic over an extensive area. It is impossible to quantify either frequency or exposure. The risk is believed to be very small.

(vii) Was it accepted that the ash was dumped extensively throughout the reclamation sites and that during dumping no satisfactory record or map of the dumping had been kept?

When the site first opened in 1986 the ash was co-mixed with incoming inert material as the site filling progressed. In 1986, the method of filling changed and the ash was placed only above mean high water spring level. The filling procedure was further changed in 1995 when the ash was deposited, unmixed, in designated pits and within specific areas of the site thereby concentrating its location and leaving areas of the site free from any ash deposits. No formal record or map was kept in the early days, but the dumping was restricted to a small part of the site.

From the beginning of its involvement WEB has adopted a cautious and independent approach. It has had to do so because investors would not be prepared to rely solely on the reassurances of the States who carried out the fill operation and who, in the eyes of the investors, have a vested interest. In 1995 therefore, WEB commissioned WRc Alert to investigate the whole of the West of Albert site in order to provide information on the scale and nature of the infill. This detailed report identifies the extent of the contamination and other geotechnical issues arising from the method of infill adopted.

Every developer on the Waterfront has been given a copy of the WRc report. Each developer has had to satisfy itself as to the suitability of the site for its intended purpose. Neither the States or WEB warrant the suitability of the site. The developer has then had to provide a method statement detailing how the ash will be managed. This method statement is built into the contract.

Even where the States are their own developer, as in the case of the Albert Pier Housing, the WRc Alert report has been provided to our independent engineering consultants who must then form their own view, take their own samples and recommend their own method for dealing with the contamination issues. The recommended methodology then forms part of the contract.

In this way, whether the site is being developed for public or private use, the responsibility and the liability for insuring that the issues of site stability and contamination are addressed rests with the developer and the design team and not the States.

(viii) Was it accepted that the site would be classified as contaminated land in the United Kingdom?

The Waterfront land has been used for the deposit, by burial, of concentrated incinerator ash and asbestos cement in numerous areas across the site for which there is no accurate record of deposit. This material has contaminants at levels above the recognised standards for contaminated land such as UK ICRCL and Dutch Intervention Standards. The UK definition of contaminated land as included in section 147 Environmental Protection Act 1990 as amended by the Environment Act 1995, is "any land which appears to the local authority in whose area it is situated to be in such a condition, by reason of substances in on or under the land that: (a) significant harm is caused or there is significant possibility of such harm being caused; or (b) pollution of controlled waters is being or is likely to be caused" Harm can be caused: to humans, to ecological systems, to buildings or to livestock or crops.

The whole Waterfront site would fall within the definition of contaminated land as described above. However there has been clear delineation of the site into discreet development areas, and these would have to be treated as separate sites for the purposes of such classification.

(ix) Was the States of Jersey legally, morally or ethically culpable for the health hazard and environmental threat posed by the site and had the States breached a duty of care by allowing this situation to develop?

(to be answered by the meeting of Presidents)

(x) Did the marine pollution threat posed by the site breach previous and current water pollution laws?

Since 1979, the Public Services Committee has administered 2 laws that deal with water pollution of environmental waters. They are the 'Sewerage (Miscellaneous Provisions) (Jersey) Law, 1979' and the 'Water Pollution (Jersey) Law, 2000.'

The Sewerage (Miscellaneous Provisions) (Jersey) Law, 1979 gave effect to the Convention for the prevention of Marine Pollution from Land-based Sources, which had been signed by the UK Government in 1974. It introduced a system of licences to control the discharge of sewage, trade effluents and certain prescribed substances into the sea. (This Law was repealed on the enactment of the Water Pollution (Jersey) Law, 2000 on the 27 November 2000.)

Under Article 2(1) of this Law, it was an offence for any person who discharged or caused or permitted to be discharged onto the seashore or into the sea or into any watercourse which drained into the sea by any means whatsoever any substance mentioned in Part I, II or III of Annex A of the Convention. Annex A prescribed substances including organo-halogen compounds and certain metals that are present in incinerator ash.

However the Law was flawed, impossible to administer and was totally ineffective in pollution control. It was repealed upon introduction of the Water Pollution (Jersey) Law, 2000, which came into force on the 27 November 2000. This Law provides for the control of pollution in Island waters and the implementing of provisions of the Convention for the protection of the Marine Environment of the North-East Atlantic (the OSPAR Convention) concluded in Paris on the 22 September 1992. In this Law, 'controlled waters' include the Island's coastal waters and territorial seas up to the 12-mile limit.

In this Law, 'pollution' includes the introduction directly or indirectly into controlled waters of any substance, or energy, where its introduction results or is likely to result in a hazard to human health or water supplies, harm to any living resource or aquatic eco-system, damage to any amenity or interference with any legitimate use of controlled waters. This definition is almost identical to the wording in the OSPAR Convention.

This Law has proved to be effective in pollution control and several case files have been submitted to the Attorney-General's office, which have resulted in successful prosecutions.

None of the data that has been accrued to date from the comprehensive monitoring programmes that have been carried out over many years has convinced officers of the Water Resources Section that pollution of controlled waters, as defined in this Law, has occurred as a result of the deposition of incinerator ash in the La Collette Reclamation Site. However, it is accepted that some of the components of the ash must have leached and continue to leach out of the site, but there is a huge dilution factor in the sea.

In carrying out its functions under this Law, the Committee must have regard, as far as is reasonably practicable, to the best techniques that are for the time being available and the best environmental practice that is for the time being recognised, a precautionary principle in respect of pollution and a cost principle in respect of pollution.

It would not be appropriate for the Committee to issue a Discharge Certificate under Article 26 of this Law for the operation of the reclamation site. However, a Waste Management Certificate will be needed under the proposed Waste Management (Jersey) Law, which will set out standards for 'environmentally sound management of waste', which is a requirement of the Basel Convention.

[Note: Under Article 5(a)iii of 'The Food and Environmental Protection Act 1985 (Jersey) Order, 1987, which is administered by the Harbours and Airport Committee, a licence is needed for the deposit of substances or articles with the Bailiwick either in the sea or under the seabed from a structure on land constructed or adapted wholly or mainly for the purpose of depositing solids in the sea. The need for a licence will depend on the definition of 'sea' and what is meant by a structure. Advice on this is obtainable from Jersey Harbours.]

(xi) The report by ERM suggested the possibility of increased leachate from the site in the future due to the changing pH levels. If this occurred would it pose a threat to the south-east coast and fishing in the area given the possible release of significant quantities of toxic heavy metals, for example cadmium?

This will always be a possibility. However the process would be a slow one and with the levels of ash remaining in the site at this stage (even at the beginning) the rate of leaching would be such that no human health risk would arise. Dilution in the sea would be considerable and even marine organisms would be unlikely to ingest toxic levels. Current monitoring provides a means of assessing this (potential) process at all times.

(xii) Notwithstanding the known marine pollution potential of the ash, why had infill excavated from the West of Albert site, contaminated with the ash, been dumped with the ordinary infill into the tide permeable land reclamation tipping zone of the La Colette 2 site?

This has not occurred. Any fly ash contamination moved to La Collette has been placed in specially constructed containment structures.

(xiii) Did the dumping of the ash in a tide permeable site, and consequently the possible release into the marine environment of many of its hazardous components, put the Island in breach of international obligations; for example, OSPAR?

No. OSPAR does provide for best environmental practice procedures to be adopted, e.g from 1987 all ash was dumped above the mean high water level.

(xiv) What were the hypothetical 'legal' grounds for not having a public register of contaminated land?

P&E Officer discussions on contaminated land policy and response have included the possibility of the Department drawing up a list of known and potentially contaminated sites.

Applications for the development of land are currently checked against spatial data such as Island Plan Zones and other relevant land-use designations. Sites where contamination may be a hazard and could be checked in this way and, if necessary, appropriate action taken.

However it was also identified that some careful thought would need to be given to how this is implemented to ensure that the Committee was not placing itself in a difficult legal position by gathering and holding such potentially contentious information.

It was felt that the principal risk would be the potential for legal action based on unfair devaluation of private land due to incorrect or unjustified registration of land as 'contaminated'. We were also conscious that the UK contaminated land register, which all Local Authorities were required to draw up under Section 143 of the 1990 Environmental Protection Act, was withdrawn for a number of reasons including the potential problem of property blight.

One solution considered was for the Department to maintain this information on a confidential basis. This was thought to be inappropriate as it is likely to be the type of information that we would be duty bound to share under the Code of Practice on Access to Official Information. It was also suggested that the Department might be obliged to make the information available when responding to legal search requests on property.

It was felt that the potential problems identified were not insurmountable but it would be prudent to seek legal advice to be sure that the implications of setting up a register or inventory were clear and that proper safeguards were in place to avoid such complications.

This advice has recently been received from the Solicitor General. The indications are that the problems can be overcome through use of suitable legal disclaimers. It was also made clear that careful thought would need to be given to how sites were classified, on what grounds, and the source of the information used.

The options will continue to be explored through the current Officer working group.

(xv) What was meant by public access to 'relevant information about contaminated sites'?

Clearly there are situations where certain information is deemed inappropriate for the wider public domain. This is especially true for information related to human health or the wider environment where often data needs to be interpreted carefully and with scientific rigour to avoid incorrect conclusions being made over risks.

The legal advice received on the implications of operating a register system has confirmed that the form it takes and just what information is held and made available needs to be carefully considered.

Currently in the UK the Environment Agency and Local Authorities are required to maintain a register of contaminated sites under the new Contaminated Land Regulations. Information relating to registered sites must be made available to members of the public but there are certain exemptions.

In developing a local register, it is reasonable to expect that whilst it should be available to the public, there are likely to be restrictions of some form on access to the information it holds. Access levels for planners, lawyers, professionals, and the public will have to be defined.

(xvi) Did the planning department have a register of contaminated land?

No. For the reasons set out in the answer to question 14, the Committee has not implemented a formal register of this nature.

The Department does however hold information on a number of sites where contamination currently is, or has been, an issue. Examples include sites where reports have been submitted by developers which provide the results of desk studies, site investigations, risk assessments and remediation strategies.

Various investigations have also been commissioned for the States on the pollution risks from historic landfill sites.

Other generic records exist of land uses that may lead to residual contaminants being present in the ground such as refuelling stations, industrial sites etc.

(xvii) Had the ash or excavated rubble contaminated with the ash - at any time - been dumped elsewhere in the island and, if so, where?

No - all contaminated material has either been contained on site or removed to La Collette II for disposal in the designated ash pits. The extra cost of this specific disposal method has been included in contact prices and paid to the contractor. The recent reported use of ash from a school at St Ouen's was shown to be incorrect.

(xviii) What would be defined as 'satisfactory remedial action', for example a clean up sufficient to allow a proposed construction and use; a clean up to allow all future constructions and use; a clean up to ensure long-term public safety; a clean up to ensure permanent protection of the environment from the contamination?

The term "satisfactory remedial action" may include all or any combination of the examples mentioned dependent on this type and scale of contaminants present on the land in question, and whether the contaminants are likely to become mobile.

Remedial action designed to achieve fitness for use my not preclude the need for further action at a later date to satisfy a subsequent change in land use, this is one of the reasons to have a contaminated land register, to monitor and record remedial action.

(xix) The gaseous emissions from municipal waste incinerators had long been recognised in the literature as a human health hazard. Was it therefore acceptable of the States to build new housing estates and schools within a couple of hundred metres of the incinerator stack?

The concept of controls for emissions from new incineration plant are based on the following two criteria:

 To use of best available techniques to remove as far as is reasonably practicable as much of the noxious and toxic elements of the flue gas prior to emission to atmosphere, ii) To ensure that through adequate stack height and efflux velocity the residual emissions of pollutants in the flue gas after treatment are so diluted, that on grounding of the plume the local air quality is in compliance with current EU air quality standards and does not give rise to nuisance.

It is unlikely that properties within a couple of hundred metres of the base of the chimney stack will be close to the point of grounding of the plume which may occur as little as twice the stack height in distance but can often be far greater. However, properties in close proximity to any industrial site are likely to be subject to adverse levels of noise from plant and traffic, and fugitive emissions from the handling, transport and processing of raw materials and waste products.

JDH 25 February 2002

Contaminated sites responses to Sen. Syrret February 2002

Appendix 3

APPLIED AND ENVIRONMENTAL MICROBIOLOGY, Mar. 1999, p. 1092–1098 0099-2240/99/804.00+0 Copyright © 1999, American Society for Microbiology. All Rights Reserved. Vol. 65, No. 3

Enhanced Bioaccumulation of Heavy Metal Ions by Bacterial Cells Due to Surface Display of Short Metal Binding Peptides

PAVEL KOTRBA,1 LUCIE DOLEČKOVÁ,2 VÍCTOR DE LORENZO,3 AND TOMAS RUML1+

Department of Biochemistry and Microbiology, Institute of Chemical Technology, 166 28 Prague, ¹ and Department of Biochemistry, Institute of Organic Chemistry and Biochemistry CAS, 166 10 Prague, ² Czech Republic, and Centro Nacional de Biotecnologia, CSIC, 28049 Madrid, Spain²

Received 7 August 1998/Accepted 18 December 1998

Metal binding peptides of sequences Gly-His-His-Pro-His-Gly (named HP) and Gly-Cys-Gly-Cys-Gro-Cys-Gly-Cys-Gly (named CP) were genetically engineered into LamB protein and expressed in Escherichia coli. The Cd²+-to-HP and Cd²+-to-CP stoichiometries of peptides were 1:1 and 3:1, respectively. Hybrid LamB proteins were found to be properly folded in the outer membrane of E. coli. Isolated cell envelopes of E. coli bearing newly added metal binding peptides showed an up to 1.8-fold increase in Cd²+ binding capacity. The bioaccumulation of Cd²+, Cu²+, and Zu²+ by E. coli was evaluated. Surface display of CP multiplied the ability of E. coli to bind Cd²+ from growth medium fourfold. Display of HP peptide did not contribute to an increase in the accumulation of Cu²+ and Zu²+. However, Cu²+ ceased contribution of HP for Cd²+ accumulation, probably due to the strong binding of Cu²+ to HP. Thus, considering the cooperation of cell structures with inserted peptides, the relative affinities of metal binding peptide and, for example, the cell wall to metal ion should be taken into account in the rational design of peptide sequences possessing specificity for a particular metal.

During the last few decades extensive attention has been paid to the hazards arising from contamination of the environment by heavy metals (35). Decontamination of heavy metals in the soil and water around industrial plants has been a challenge for a long time. The use of microorganisms for the recovery of metals from waste streams (15, 17, 30, 31), as well as the employment of plants for landfill application (47), has achieved growing attention. Lower cost and higher efficiency at low metal concentrations make biotechnological processes very attractive in comparison to physicochemical methods for heavy metal removal (17).

heavy metal removal (17).

The microbial processes for bioremediation of toxic metals and radionuclides from waste streams employ living cells, non-living biomass, or biopolymers as biosorbents (17, 30, 46). Specific metabolic pathways resulting in bioprecipitation of heavy metals or their biotransformation to less toxic or easily recoverable forms have been described (15, 17, 30, 31). A wide variety of fungi, algae, and bacteria are now under study or are already in use as biosorbents for heavy metal remediation (17, 30, 46). Metal binding by biomolecules of structural components or excreted polymers is fortuitous, and relative efficiencies depend on attributes of the metal ion, as well as on the reactivity of the provided ligands. The macromolecular composition of biosorbent could be manipulated by cultivation conditions (e.g., stress-inducible fungal melanins [30]) to improve its metal binding properties.

prove its metal binding properties.

The principles governing the selectivity of biomolecules for metal ions are described by semiempirical and qualitative theories, such as the HSAB (hard and soft acids and bases) principle and the Irwing-Williams series of stability constants for divalent ions (26). Anchoring of particular amino acid sequences to biosorbent material could contribute to the selec-

A number of vehicles, including subunits of cellular appendages or outer membrane proteins, are now in use for the display and action of enzymes, peptide libraries, antigenic determinants, or single-chain antibodies on the surface of gramnegative bacteria (19). The Escherichia coli maltoporin (LamB) has been well characterized. The LamB protein is a trimeric outer membrane (OM) protein of E. coli sustaining two biological functions. It is used as a surface receptor by a number of coliphages, including phage \(\lambda \) (6, 12, 14), and participates in the transport of maltose and maltodextrins across the OM (45). LamB tolerates insertions of long heterologous peptides at a permissive loop (between structural codons 153 and 154) exposed to the external medium without a loss of function (5, 9, 11, 13, 21, 43, 44). Successful attempts to introduce polyhistidine tails (41), as well as yeast and human metallothioneins (42), to LamB have been reported. The surface display of the polyhistidine tail and/or metallothioneins led to a significant increase in the accumulation of divalent heavy metal ions. The LamB protein was also used to search for repeating peptides responsible for a specific adhesion of E. coli to gold, chromium, or iron oxide (7, 8).

We examined metal binding properties of E. coli strains displaying short peptides as a fusion to LamB protein. The histidine-rich sequence Gly-His-His-Pro-His-Gly employed in this study was named HP. HP represents one to three multiple repeats along the C-terminal part of the human plasma metal transport protein known as the histidine rich-glycoprotein

tivity for specific metal ions. Biosorbents could be enriched with amino acids classified by HSAB principles to be stronger ligands of transition metals than those naturally present on the microbial surfaces (26, 30). The principal benefit of selectivity should provide preference of particular metal ions for its specific coordination preferences (1), exploiting peptides with known fold. Surface exposure of metal binding peptides could improve metal binding properties of microorganisms employed in various systems based not only on biosorption but also on the metabolic activities located on the cell surface (15, 31).

Ocrresponding author. Mailing address: Department of Biochemistry and Microbiology, Institute of Chemical Technology, Technická 5, 166 28 Prague, Czech Republic. Phone: (420) 2-24353022. Fax: (420) 2-311990. E-mail: TOMAS.RUML@VSCHT.CZ.

(HRG) (28). The HRG binds heme and various divalent heavy metal ions with the following apparent order of affinity: $Cu^{2+} \sim Hg^{2+} > Zn^{2+} > Ni^{2+} > Cd^{2+} > Co^{2+}$ (35). The HP sequence is believed to form surface metal binding sites (MBSs) of HRG, and it has been also successfully used to immobilize Cu²⁺ and Zn²⁺ on IMAC columns (27). The cysteine-rich amino acid sequence, named CP (Gly-Cys-Gly-Cys-Pro-Cys-Gly-Cys-Gly), was previously selected in our labora-tory as a result of screening of synthetic peptides consisting of cysteine and histidine residues for Cd²⁺ binding (29). CP was further characterized and employed for display on the E. coli

MATERIALS AND METHODS

Chomical synthesis of poptides. The standard Meerifield solid-phase technique with discopropylcarbodilmide-1-hydroxybemoristical activation chemistry was used for the synthesis of the CP peptide of the sequence AOCGip-Oys-Fre-Oys-Gip-Oys-Fre-Oys-Gip-Oys-Fre-Oys-Gip-Oys-Fre-Oys-Gip-Oys-Fre-Oys-Gip-Oys-Fre-Oys-Gip-Oys-Fre-Oys-Gip-Oys-Fre-Oys-Gip-Oys-Fre-Oys-Gip-Oys-Fre-Oys-Gip-Oys-Fre-Oys-Gip-Oys-Fre-Oys-Gip-Oys-Fre-Oys-Gip-Oys-Fre-Oys-Gip-Oys-Fre-Oys-Gip-Oys-Fre-Oys-Gip-Oys-Fre-Oys-Gip-Oys-Fre-Oys-Gip-Oys-Fre-Oys-Gip-Oys-

mM DTNB in 0.1 M phosphane buffer (pH 7.27) for 1 h with shaking. The absorption was read at 4.12 mm (s_{all} = 13,700 M⁻²) m⁻¹) after the completed reaction.

Strains, plasmids, general procedures, and modia. E. col/TG1 (supE had 5 this Affect phosphane) and the completed reaction.

Strains, plasmids, plasmids. The R had B dez AMLS1] was used to host and multiply recombinant plasmids. The hams mutant E. coll strain pope530 (supE the hams B this lac 17 reted det.5 world) was used as a recipient of all expression vectors bearing LamB variants. The R to yet of lipopolysaccharide (LPS) of this strain was determined by stiver staining (21). E. col LES92 (supE supErS BadE514 gaRE2 galT22 motil 1 typE55 fac(T) was used to propagate \(\text{A}\) phages. Lambda phages \(\text{A}\) '(wild type), \(\text{A}\) '(single mutant), and \(\text{A}\) '(double mutant) have been desorbed elsewhere (6, 12). The vector pLEBS used for the expression of LamB variants has been desorbed previously (9), pLEBS is a decivative of pSC101-based, chloramphenicol (Cm)-resistant vector pVDLB carrying a promoterless towall-153 gene that is expressed through the Plac promoter of the vector. Insertions between the positions 153 and 154 of the amino acid requence of LamB protein have been constructed via a unique BawHI site of the plasmid. Recombinant DNA techniques were carried out according to a standard percent of the based on the superior of the state of the superior of the section of the superior of the section was verified by restriction analysis, and positive dones were subjected to DNAs sequencing.

Minimal MIS medium (12.5 mM HEPES, pH 7.1; 50 mM MnCl; 20 mM NRCl; 30 mM NRCl; 20 mM

5'-GATCCAGCAGGCTGCGGTTGTCCCATGGGGTTGTGGCGCT-3' [plus strand]) encodes the N-Ala-Gly-Cys-Gly-Cys-Gly-Cys-Gly-Cys-Gly-Cys-Gly-Ala-C sequence, which has been named CP. By using this approach, DNA fragments encoding HP peptide and CP peptide were inserted into hand-153 of the vector DEBP, resulting in hand-HP [clasmid pt_BHP] and hand-CP [clasmid pt_BCP], respectively. Subsequently, the next DNA fragments encoding HP peptide and CP peptide were inserted into hand-HP, resulting in hand-HP2 [clasmid pt_BHPCP], respectively (see Fig. 2a).

Expression of LamB derivatives (those with a genetic insertion of a metal binding size are further called LamB-MBS) was performed in MJS or LB media supplemented with 30 μg of Cm per ml and 100 μM IPTG.

Somstivity of E, coll to λ phage variants. High-tirer phage lysates were prepared by infection and lysis of the permissive strain E, coll LE302 as reported elsewhere (40, 42). Approximately $100 \mu l$ of lysate (titer of $\sim 10^{50}$ PFU/ml) was streaked in a line across the surface of LB agar plates (supplemented with 5 mM (each) CAC₂ and MgSO₂, θ and θ of the primary of Mi PTG) and allowed to dry, E, coll pope 510 bearing each of the plasmids encoding LamB variants was then streaked perpendicular to and across the phage line in a single swatch. The sensitivity was evaluated after overnight incubation at 37°C.

Proparation of E, coll convelopes. The method for the preparation of cell envelopes of E, coll sope 510 expressing LamB or LamB-MSEs was harvested from 500 ml of LB media supplemented with 50 μ gof Cm per ml and 100 μ M HTPG. The pellet was resuspended in 5 ml of 50 mM HEPES (pH 7.2), and the cells were distintegrated by using X-press (LXE), Stockholm, Sweden). Unbroken cells were removed by low-speed centrifugation (1,500 × g for 10 min at 4°C). Supermatant (disrupted cells) was incubated with Rhase (100 μ Mm) and DNase (50 μ Mm) in the presence of MgCl₃ (5 mM). Envelopes were separated at 48,000 × g for 30 min at 4°C, washed five times with 6 ml of ice-cold demineralized water, and then freeze-dried.

We also used Percoll gradient separation as described before (33) for the

alized water, and then freeze-dried.

We also used Percoil gradient separation as described before (33) for the
small-scale preparation of OM and funer membrane (IM) fractions in order to
determine the localization of LamB-MES proteins.

Protein techniques. Whole-cell extracts and/or equivalent portions of cell
envelopes, membrane preparations, or cytoplasmic fractions were examined by
electrophoresis in a denauting polyacipanide gel. Proteins were alternatively
electroblotted on nitrocellulose membranes blocked with 10% skim milk in TES

Protein techniques. Whole-cell extracts and/or equivalent portions of cell envelopes, membrane preparations, or cytoplasmic fractions were examined by electrophoresis in a denaturing polyacoplandia gel. Proteins were alternatively electrophoresis in a denaturing polyacoplandia gel. Proteins were alternatively electrophoresis in a denaturing polyacoplandia gel. Proteins were alternatively electrophoresis in a denaturing polyacoplandia gel. Proteins were alternatively electrophoresis in a denaturing of the control of the contr

RESULTS

Stoichiometry of Cd2+ binding to synthetic peptides. Two peptides predicted to be candidates for the engineering of peptides predicted to be candidates for the engineering of bacterial surface for enhanced heavy metal binding were syn-thesized. The amino acid sequences were Gly-His-His-Pro-His-Gly (i.e., HP) and Gly-Cys-Gly-Cys-Pro-Cys-Gly-Cys-Gly (i.e., CP). The correct amino acid composition and the amount of peptide bound on TentaGel resin were evaluated by amino

KOTRBA ET AL. 1094 APPL ENVIRON, MICRORIOL.

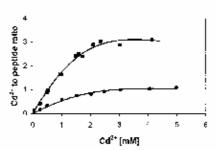


FIG. 1. The Cd^{2+} -to-peptide stoichiometry expressed as the plot of initial Cd^{2+} concentration against the complexed Cd^{2+} -to-peptide molar ratio. A total of 0.6 $_{\rm p}$ -mol of CP peptide (aguzes) and/or 1.2 $_{\rm p}$ -mol of CP peptide (declas) were incubated on TentaGel resis in 1 ml of Cd^{2+} containing 30 mM Tels-Cl (pH 7.4). In the case of HP peptide metal binding studies, 0.2 M ENO_2 was added as a background electrolyte. The portion of unbound Cd^{2+} was determined by atomic absorption spectrometry.

acid analysis after total peptide hydrotysis. The sulfhydryl content of CP peptide was also determined. These analyses confirmed that peptides were synthesized as correct full-length sequences. The amounts of peptide were found to be 138 and 118 nmol per mg of carrier for HP and CP peptide, respectively. The yield represented approximately 50% of the theoretical yield.

tively. The yield represented approximately 50% of the theoretical yield.

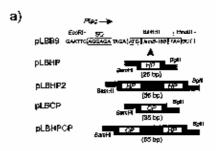
The Cd²⁺-to-peptide stoichiometry was determined from the plot of the initial metal concentration against the molar ratio of bound cadmium to peptide (Fig. 1). The resulting Cd²⁺-to-HP peptide stoichiometry of 1:1 indicated the presence of a single metal binding site. On the other hand, CP peptide was found to bind three equivalents of Cd²⁺. These data suggested that both HP and CP peptides provide potent MBSs. MBSs.

Expression of LamB hybrid proteins. DNA fragm coding predicted MBSs were engineered into the lamB-153 gene at a permissive position equivalent to the protein loop game at a permissive pushed requirement to the protein local expression vectors carrying the chimeric gene lamb-mbs were named pLBHP (one HP sequence), pLBHP2 (two HP sequences), pLBCP (one CP sequence), and pLBHPCP (a combination of LP sequence).

HP and CP sequences).

The LamB-MBS proteins were expressed in lamB-defective E. coli pop6510 as full-length products, with no signs of proteolytic degradation, as demonstrated by immunoblot analysis of the crude cell extracts with anti-LamB serum (Fig. 2b). The growth rate of cells expressing LamB-MBS was not altered compared to cells expressing LamB during the exponential phase. However, a slight depression of the biomass yield (about 20%) was observed with cells expressing LamB-CP and LamB-HPCP.

Localization of LamB-MBS and functional tolerance of LamB to specific insertions of HP and CP sequences. To address the issue of the effect of insertion of HP and CP sequences and/or their combination on the targeting of Lamb-MBS into outer membrane, we fractionated envelopes of E. cali pop6510 expressing Lamb-MBS. We noted a sharp, white, can popo510 expressing Lamb-MISS. We noted a sharp, wines, high-density band resulting from Percoil gradient centrifuga-tion of the disintegrated cells corresponding to the OM of E. coli (33) (Fig. 3a). A band close to the top of the gradient as a fraction consisting of the IM was also separated out. The ma-jority of the Lamb-MBS proteins were present in the fraction



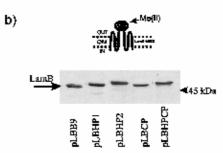


FIG. 2. (a) Organization of the lawsB-salar gene within the pLEB9 expression vector (a derivative of the low-copy-number vector pVDL3 bearing lawsB-173 gene expressed throughout the lac pecanotes). The orientation of the promoter is marked by an acrow. The orientation of the promoter is marked by an acrow. The orientation of the promoter is marked by an acrow. The orientation of the promoter relevant to the specific generation is extended as the interest of the promoter relevant to the specific generation for salar shallows and compositions of the MBSs see Table 1. (b) Expression of LamB-MBD in E cal popts 10. Crodic extracts of approximately 2 × 10° orien of LamB-MBD in E cal popts 10. Crodic extracts of approximately 2 × 10° orien of LamB-MBD in E cal popts 10. LamB vector in a decay or the control of the control orientation or orientation of the control orientation or orientation orientation orientation or orientation orienta

corresponding to the OM of E. coli, i.e., at the site of its natural

corresponding to the OM of E. coli, i.e., at the site of its natural destination (Fig. 3 shows LamB-HP2 as an example).

The sensitivity of E. coli pop65 10 expressing LamB-MBS to lambda phasps \(\hat{h}^+\) (wild type), \(\hat{h}^0\), and \(\hat{h}h^+\) was determined in order to evaluate the effect of specific insertions (MBSs) on the folding of the LamB protein. No changes of the sensitivity to lambda phases were detected in the chimeric LamB.

Metal binding properties of isolated envelopes containing LamB-MBSs. Cell envelopes were prepared by a method described elsewhere (2). The presence of the LamB-MBSs was determined by immunoblot analysis. No significant differences in LamB-MBS content were observed among all of the prepdetermined by immunoblot analysis. No significant differences in LamB-MBS content were observed among all of the preparations (data not shown). We did not detect any contamination of the envelopes with nucleic acids. The reaction of the envelopes with Cd¹⁺ was performed as described in Materials and Methods in an arrangement similar to that described else-where (2, 23, 24), but the pH was set to 7.0. This value re-mained unchanged during the reaction. As shown in Table 1, E. coli envelopes containing any of the LamB-MBSs bound significantly higher amounts of Cd²⁺ than did those of cells

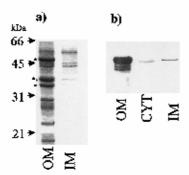


FIG. 3. Localization of LamB-HP2 protein in E. coii. (a) Solium dodecyl sulfata-polyscrylamide gel electrophoresis of the OM and the IM fractions resulting from Percoil gradient centrifugation. Proteins of resolved membrane vesicles of approximately 5 × 10⁶ cells were percipitated with acetone and run in a 12% denaturing polyacrylamide gel. Asteriaks indicate the mijor OM protein cidentified on a molecular-tale basis (LamB-HP2, 488 EOx; OmpC, 382 EOx; OmpF, 37.0 EOx; OmpA, 35.1 EOx). (b) Immunothemical detection of LamB-HP2 in the same membrane fractions. CYT, aliquet portion of cytoplasm resulting from high-speed centrifugation of disrupted cells.

expressing "wild-type" LamB protein. The best metal binding expressing which type Lamb protein. The best metal bribing capacity showed envelopes containing Lamb-HP2 and/or Lamb-CP, which exceeded the natural ability of E. coli cell envelopes to bind Cd²⁺ by 1.8-fold.

Metal binding properties of E. coli displaying Lamb-MBS. The increased Cd²⁺ binding capacity of the manipulated cell

wall of E. coli led us to evaluate the influence of specific genetic insertions on metal binding by viable E. coli cells. The

genetic insertions on metal binding by viable $E.\ coli$ cells. The accumulation of bivalent metal ions in the "single metal" $(Cd^{2+},\ Cu^{2+},\ cu^{2+})$ system was compared to that of the cells expressing wild-type LamB protein.

As shown in Fig. 4, cells displaying LamB-MBSs accumulated Cd^{2+} with an efficiency higher than that of the other tested metals. Insertion of MBSs containing both histidines (HP) and cysteines (CP) into the LamB protein led to the increase of the amount of accumulated Cd^{2+} from media supplemented with 30 μ M Cd^{2+} . A more than twofold increase (2.2 ± 0.3) of Cd^{2+} bioaccumulation was observed with LamB-HP. Duplication of the HP sequence (LamB-HP2) led to the additional increase (3.1 ± 1.0) times) of the amount of accu-

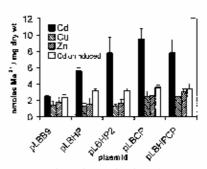


FIG. 4. Bioaccumulation of heavy metal ions by E. coti pop6510 expressing LamB-MBSs in a single-metal system. E. coti transformed with plasmids carying specific insertions in lamB-J53 (see Table 1) or the control plasmid pt.BB9 was grown in MJS medium and induced at OP₅₀₀ = 0.3 with IPTG except for the control uninduced cells (open bars). Cells were further grown until OD₅₀₀ = 0.4, and then a 30 µM concentration of Cell., Coll., or Zoci., was added. The metal content was determined by atomic absorption spectrements after an additional 3.5 h of cultivation. The bars represent the mean value of three to five independent experiments.

mulated Cd^{2+} . A nearly fourfold increase (3.8 \pm 0.8) of the bioaccumulation occurred in the cells expressing LamB-Cd²⁺ bioaccumulation occurred in the cells expressing Lamb-CP. However, there was no additive effect of the combination of HP and CP sequences (Lamb-HPCP) on the total amount of accumulated Cd²⁺ (Fig. 4). A similar pattern was found for the accumulation of Cd²⁺ from medium supplemented with 15 μM Cd²⁺ (Fig. 5). A slight increase in the Cd²⁺ bioaccumu-

lation by uninduced cells was observed (Fig. 4). This was due to leaking expression of LamB-MBS as detected by immunoblot.

E. coli cells preadsorbed with Cd²⁺ were incubated in excess of EDTA (see Materials and Methods) in order to evaluate the portion of Cd²⁺ bound to the surface structures. Such treatment which may also cause a partial release of LPSc (21). portion of Cd⁻⁺ bound to the surface structures. Such treat-ment, which may also cause a partial release of LPSs (31), resulted in removal of 50 to 60% of the total Cd²⁺ accumu-lated by cells expressing LamB-MBSs (Table 1). The surface display of the histidine-based MBS (LamB-HP and LamB-HP2) did not enhance the bioaccumulation of both Cu²⁺ and Zn²⁺ in contrast to the accumulation of Cd²⁺. A slightly increased bioaccumulation of Cu²⁺ and Zn²⁺ was observed with cells expressing Lamb-CP and Lamb-HPCP. However, this increase was less than twice that of the control.

TABLE 1. The amount of Cd^{2+} bound by envelopes of E, coli pop6510 expressing LamB-MBSs and a portion of Cd^{2+} removed from the surface by EDTA treatment^a

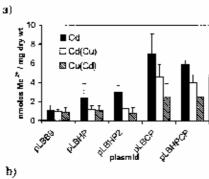
Plasmid	Amino acid sequences (MBSs) inserted into LamB protein ^a	Od ²⁺ removed	Cd ²⁺ bound by
transformed		by EDTA	cell envelopes
to E. coli		treatment	(nmol/mg
pop6510		(%)"	[dry wt]) ²
pLBB9	None	69	125
pLBHP	N.Asp.Pro.Ah.Gly <u>.His.His.Pro.His.Gly</u> .Ala.C	60	196
pLBHP2	N.(Asp.Pro.Ah. <u>Gly.His.His.Pro.His.Gly</u> .Ala) ₇ .C	59	226
pLBCP	N.Asp.Pro.Ah.Gly.Oys.Gly.Oys.Pro.Oys.Gly.Oys.Gly.Ah.C	55	227
pLBHPCP	N.Asp.Pro.Ah. <u>Gly.Oys.Gly.Oys.Pro.Oys.Gly.Oys.Gly</u> .Ah.Asp.Pro.Ah. <u>Gly.His.His.Pro.His.Gly</u> .Ala.C	51	184

"Amino acid sequences corresponding to DNA fragments artificially introduced into lame 1-153 (MBSs) and the names of the corresponding plasmids are indicated.

The amino acids of the MBSs are underlined.

Cells preadsorbed with cadmium as described in the legend to Fig. 3 were treated for 15 min with an excess volume of ice-cold 5 mM EDTA in 0.85% NaCl (pH 7.1), and the metal content was determined by atomic absorption spectrometry. The data represent the mean value of two independent experiments with a span of less than 10%. First, 3 mg (day wt) of the cell envelopes was incubated for 30 min in 3 ml of 5 mM CdCl₂ in 25 mM HEPES (pH 7.0). The metal content of the envelopes was then determined by atomic absorption spectrometry.

1096 KOTRBA ET AL. APPL ENVIRON. MICROBIOL.



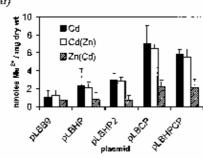


FIG. 5. Bioaccumulation of heavy metal ions by E. coli pope518 expressing Lamb-MBSs in the double metal system. E. coli transformed with plasmids earying specific insertions in Assab-173 (see Table 1) to the control plasmid pLEB9 was grown in MJS medium and induced at $OD_{300} = 0.3$ with HPTG. Cells were than grown until $OD_{300} = 0.4$ and supplemented with 15 μ M Cell_2 and/or equimals: mixtures (15 μ M concentrations of each metal ion) of either $Cell_2$ and $Cell_2$ and $Cell_3$ and $Zell_4$ (b). Their metal context was determined by atomic biospection spectroenery after a notification 35 h of cultivation. The second metal of the mixture is indicated in brackets. The bass represent mean value of three independents experiments.

The absence of a contribution from the HP sequence for the Cu²⁺ binding was quite unexpected since the imidazotium group has been described as a ligand with a relatively high affinity for Cu²⁺ in biological systems (26). The competition of Cd²⁺ with Cu²⁺ or Zn²⁺ for LamB-MBSs in vivo was evaluated in order to elucidate this phenomenon. As shown in Fig. 5a, the amount of Cd²⁺ accumulated by cells expressing LamB-HP and LamB-HP2 from media containing an equimolar mixture of Cd²⁺ and Cu²⁺ (15 µM) caused less than a 10% decrease of bioaccumulation of Cd²⁺ by cells displaying HP sequences compared to the same cells grown in the presence of a single 15 µM Cd²⁺ (Fig. 5b). The cells expressing LamB-CP and LamB-HPCP accumulated a greater amount of heavy metals than the control from equimolar mixtures of both Cd²⁺ and Cu²⁺ (Fig. 5) However, the decrease in the amount of Cd²⁺ accumulated due to the presence of Cu²⁺ in the medium was significant (Fig. 5a). An apparently lower effect of Zn²⁺ on Cd²⁺ accumulation was observed with cells displaying the CP sequence (Fig. 5b).

DISCUSSION

The introduction of additional peptides serving as heavy metal ligands on the microbial surface represents one possible way for improving the metal binding properties of the biomass in terms of capacity, kinetics, and selectivity. The metal binding ability of the E coli cell wall has been studied in detail previously (2, 16, 23, 24). The specific native groups of the OM participating in metal binding are the polar head groups of phospholipids acting mainly at the inner layer of the OM and the acidic groups of the exposed (hydrophilic) polypeptides and at the outer half of the OM LPSs. The LPSs provide both carboxyl and phosphoryl groups as ligands. However, only the latter group is responsible for the high affinity of divalent metal ions for LPS (16). The peptidoglycan (PG) layer of E. coli, which is most probably one molecule thick, binds metal ions via the carboxyl group of the n-glutamic acid of the peptide stem and the hydroxyl groups of the glycan backbone (23). The two-step deposition process may increase the apparent metal binding capacity of PG (3, 23) and would include the stoichiometric binding of metal ion, generating a nucleation site for the subsequent precipitation of metal above the stoichiometric amounts.

amounts.

The OM LamB protein of E. coli has been reported to tolerate the genetic insertions of heterologous peptides at positions between the structural codons 153 and 154 (5, 9, 11, 13, 22, 42, 43, 44). The genetic insertions of the HP and CP sequences into lamB-153 resulted in LamB-MBS hybrid proteins located in the OM. Furthermore, the LamB-MBSs retained their biological function as a lambda phage port, indicating a maintenance of their overall folding pattern. The Cd²⁺-to-peptide stoichiometry determined for synthetic HP and CP peptides is less than one metal ion per amino acid residue possessing metal binding properties (Fig. 1). This suggests specific folding of the sequence around the metal ion(s). The geometry of the MBSs of both HP and CP peptides is currently under study.

The increase of the metal binding capacity of the E. coli envelopes was significant (Table 1). Thus, the introduction of metal binding peptides onto the surface of the microorganism to be used as nonliving material for bioremediation may improve the opposes. It could be hypothesized that such an anonyme the opposes.

The increase of the metal binding capacity of the *E. coli* envelopes was significant (Table 1). Thus, the introduction of metal binding peptides onto the surface of the microorganism to be used as nonliving material for bioremediation may improve the process. It could be hypothesized that such an approach will improve not only the metal binding capacity of the biosorbent but also the kinetics of the process. An appropriate carrier for the surface display could be the only limiting factor. The C-terminal part of o-agglutinin could be such a carrier in yeast cells (39), which are being considered for use in the bioremediation of heavy metal ions (25).

We used *E. coli* as a model to evaluate changes of the metal

We used *E. coli* as a model to evaluate changes of the metal binding properties due to surface display of the metal binding peptides. Surface display of HP and CP sequences resulted in a significant increase in Cd²⁺ bioaccumulation by growing *E. coli* (Fig. 4 and 5). The number of Cd²⁺ binding sites generated by surface display of polyhistidine or metallothionein did not fully account for the amount of accumulated Cd²⁺, as has been previously proposed (41, 42). The amount of LamB used in the expression system ranged from 1,000 to 5,000 molecules of protein per cell. This value is 2 to 3 orders of magnitude lower than the increment in Cd²⁺ accumulation due to the insertion of the MBS. It has been suggested that displayed peptides favor the interaction of Cd²⁺ with other bacterial structures by increasing the local concentration of metal ions (41, 42). The cell wall components involved could be, for example, LPSs (*E. coli* pop6510 possesses the R type of LPS), which are known as compounds that directly interact with the LamB protein (36) and provide sufficiently effective metal ligands (16). The data

on desorption-bound Cd2+ by EDTA treatment suggest that more than one-half of the metal is located on the surface.

A different situation has been observed for Cu2+ and Zn2+ bioaccumulation. While E. coli displaying CP peptide (as LamB-CP or LamB-HPCP) accumulated both Cu²⁺ and Zn²⁺ in apparently higher amounts than did the control cells ex-pressing LamB protein, the surface display of HP did not promote any increase of bioaccumulation of these two metals (Fig. 4). The apparent lack of contribution of the HP sequence (Fig. 4). The apparent lack of contribution of the HP sequence was unexpected because histidine residues possess a higher affinity to Cu²⁺ than to Cd²⁺ (26, 34) and because the HP sequence by itself has been shown to be an effective ligand for both Cu²⁺ and Zn²⁺ (27). We also did not detect any contribution of HP display to the enhanced bioaccumulation of Ni²⁺ and Co²⁺ (unpublished observations). Moreover, the presence of Cu²⁺ in the medium resulted in the inhibition of Cd²⁺ birding to really displaying HP centile. This finding confirms of Ca⁺⁺ in the medium resulted in the inhibition of Ca⁺⁺ be binding by cells displaying HP peptide. This finding confirms the strong binding of Cu⁺⁺ to the HP sequence in vivo. However, no increase of the Cu²⁺ accumulation by corresponding cells was observed. In adopting a model explaining the disproportions between the number of MBSs and the amount of accumulated Cd²⁺ (41, 42), it should be considered that such a figure could be due to the lower affinity of bacterial surface components other than that of HP to Cu²⁺. The lack of interesting the long results for a Ca²⁺ by restle displaying the country of the contract of the country of the countr crease in bioaccumulation of Zn2+ by cells displaying HP se crease in bioaccumilation of $Z\Pi^{-1}$ by calls displaying Π^{-1} sequences can be explained by the low relative affinity of cell wall components for $Z\Pi^{2+}$, which is otherwise bound to HP sequence less avidly than Cd^{2+} (Fig. 5b). Since the affinity of $Z\Pi^{2+}$ to HRG is higher than that of Cd^{2+} (33), the absence of any significant effect of $Z\Pi^{2+}$ on the bioaccumulation of Cd^{2+} by the Π^{2+} display is write inverted. due to HP display is quite interesting. It could indicate a different conformation of the HP sequence fused to LamB from that in HRG or the participation of other amino acid residues in the formation of the metal (Zn²⁺) binding site of HDG.

E. coli displaying the CP peptide (either as LamB-CP or LamB-HPCP) exhibited an accumulation of both Cd²⁺ and its counterpart in the equimolar mixture (Cu²⁺ or Zn²⁺) higher at of the control cells. The results shown in Fig. 5 are in agreement with the relative affinities of tested metals to imi-

dazolium and suffhydryl groups (26).

The LamB protein is a very attractive "broad-range" vehicle that could be efficiently expressed in various gram-negative species (9, 14, 43, 44). The engineering of metal binding peptides on the surface of environmentally acceptable gram-neg-

species (9, 14.5, 44). The engineering of metal binding peptides on the surface of environmentally acceptable gram-negative bacteria such as Ralstonia eutropha and Pseudomonas purida, which are already employed in existing systems for heavy metal bioremediation (15, 30), represents a possible application. For instance, the metal binding peptides introduced on the surface of R eutropha may aid in the process of precipitation and crystallization of metal carbonates.

The search for novel peptide sequences with attention paid to their selectivity for specific metal ions is under study in our laboratory. Several designs for artificial heavy metal binding sites have been previously reported in the literature. The synthetic peptide Boc-Cys-Pro-Leu-Cys-OMe, designed as a model for Cys-containing metal binding sites, has been shown to bind Hg²⁺, Za²⁺, and Cd²⁺ via both Cys residues (48). The Za²⁺ binding via His and Glu residues was described for a model peptide mimicking the metal binding site of the ribonucleotide reductase (49). Haymore et al. (20) identified several short chelating sequences containing His, Cys, and Asp residues which could form energetically stable chelating sites with specific metal ions. Amino acid sequences forming a stable chelating stone proper process of the conditions of the process of the proc with specific metal ions. Amino acid sequences forming a sta-ble coordination sphere around transition metals were also identified by using a combinatorial peptide library approach

(4). However, the data shown in Fig. 4 and 5 indicate that not only would the affinity of a peptide or its selectivity determine the bioaccumulation of a particular metal ion but that the "reactivity" of the cell wall (and cell compartments) would also have to be taken into account.

ACKNOWLEDGMENTS

We are particularly indebted to M. Hofnung (Institut Pasteur, Puris, Ptance) for the gift of various strains and for the anti-LamB serum. This work was funded by grants of the Grant Agency of Czech Republic (203/99(650), grant VS 90074 of the Ministry of Batucation of Czech Republic, and grants ENV4-CT95-0141 and BIO4-CT97-

REFERENCES

- Burtini, I., F. Brignati, and A. Scozzafirw. 1995. Specific factors in metal ion-maccomilecular ligand interactions, p. 31–92. In G. Berchon (ed.), Handbook of metal-ligand interactions in biological fluids, vol. 1. Marcel Delker, New York, N.Y.
- Delker, New York, N.Y.

 2. Bowardigs, T. J., and S. F. Kuwal. 1981. Elading of metals to cell envelopes of Esché-chie coll X-LA ppl. Environ. Microbiol. 42:325–335.

 3. Bowardigs, T. J., and R. G. E. Murray. 1976. Uptake and retention of metals by cell wells of Escalar adolis. J. Bacteriol. 127:1502–1513.

 4. Blanchi, E., A. Feigery, A. Wallines. N. Distance.
- nchi, E., A. Folgary, A. Wallnes, M. Micora, S. Aculi, A. Phalipas, G. tatos, R. Bazzo, R. Cortess, F. Felici, and A. Pessi. 1995. A conformabily homogeneous combinatorial peptide library. J. Mol. Biol. 247:154—
- . ulnin, J., A. Charbit, and M. Hofbung. 1986. Mutagenesis by random ker inscrition into law#8 gene of Eacho-lc/kis coll K12. Mol. Gen. Genet.

- Roulais, J., A. Charbis, and M. Hofsung. 1986. Mutagenesis by random linker insertion into lawell gene of Excherichia coll X12. Mol. Gen. Genet. 208:330–348.
 Bruun-Brauton, C., and M. Hofsung. 1986. In vivo and in vitro functional alterations of the bacteriophage lambda receptor in lawell miss one mutants of Excherichia coll X-12. J. Bacteriol. 148:345–352.
 Brown S. 1997. Metal-recognition by repeating polypeptides. Nat. Biotechnol. 18:269–272.
 Brown, S. 1992. Engineered from oxide-adhesion mutants of Excherichia coll phage lambda receptor. Proc. Natl. Acad. Sci. USA 89:8631–8655.
 Caballa, A., C. Gamnia, and V. de Lorouzo. 1996. Nondisreptive detection of catabolic promoteers of Pseadowanes publish with an artigenic surface reporter system. Appl. Environ. Microbiol. 62:214–220.
 Chanty, R. L., M. Maillit, Y. L. Li, S. L. Brown, E. F. Brown, J. S. Angla, and A. J. M. Bailar. 1997. Phytoremediation of roll metals. Curr. Opin. Biotechnol. 82:79–284.
 Chantis, A., J. C. Boulula, A. Rytor, and M. Hofsung. 1996. Probing the
- not. 8:279-284.

 11. Charbit, A., J. C. Boulais, A. Rytzr, and M. Holtung. 1996. Probing the topology of a becterial membrane protein by genetic insertion of a foreign epitope: expression at the cell surface. EMBO J. 8:3029-3037.

 12. Charbit, A., and M. Holtung. 1935. Bolation of different bacteriophages using the LamB protein for adsorption on Excharbita coli K-12. J. Virol. 53:667-671.

- Charbis, A., A. Molla, W. Sauria, and M. Robung. 1988. Versatility of a vector for expression foreign polypeptides at the surface of Gram-negative bacterin. Gene Philb.—189.
 de Vries, G. E., C. K. Raymond, and R. A. Lufwig. 1984. Extension of bacteriophage A host range: selection, cloning, and characteristation of a constitutive A receptor gene. Pecc. Natl. Acad. Sci. USA 815/090-0918.
 Didak, L. S. Van Roy, M. Margany, W. Dayan, S. Taghard, and R. Layson. 1993. Immobilization of bacteria in composite membranes and development of bulue membrane reactors for heavy metal recuperation, p. 275–293. As R. Peterson (ed.), Effective membrane processes: new perspectives. Eluwer Academic Publishers, Dordrecht, The Netherlands.
 Furrits, F. C., and T. J. Bewartigs. 1985. Site specifity of metallic ion binding in Ercherichia cell K-12 lipopolysachatele. Can. J. Miccobiol. 2025–25.
 Casti, G. M. 1992. Miccobial control of heavy metal pollution, p. 50–87. As J. C. Fry, G. M. Gadd, R. A. Herbert, C. W. Jones, and I. A. Watson-Craft (ed.), Miccobial control of pollution. Cambridge University Press, Cambridge, United Kingdom.

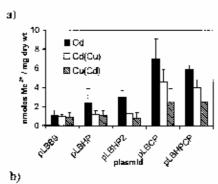
- Gelmi, M., P. Apestell, E. Cubliblo, S. Perru, L. Almsto, and A. Turnao.
 John, M., P. Apestell, E. Cubliblo, S. Perru, L. Almsto, and A. Turnao.
 John Resistance to cadmium satis and metal absorption by different microbal species. Curr. Microbiol. 20:335–341.
 Googjou, C., E. L. Foutschia, C. Sintisopoulo, and J. A. Francisco. 1993. Practical applications of engineering Gram-negative bacterial cell surfaces.
 Trends Biotechnol. 11:5–10.
 Harmston, B. L. C. and Control.
- Trends Biotechnol. Lin-10.

 20. Haymore, B. L., G. S. Bild, W. J. Salagher, N. R. Staton, and C. G. Krivl.

 1992. Introducing strong metal-hinding sites onto surfaces of proteins for
 facile and efficient metal-affinity purification. Methods (Companion Methods Enzymol.) 4:25-40.

 21. Hitchoock, P. J., and T. M. Brown. 1983. Morphological heterogeneity
 among Sainwarfth lipophysachiatide chemotypes in silver-stained polysoryiamide gels. J. Bacteriol. 154:269-277.

1096 KOTRBA ET AL. APPL ENVIRON, MICROBIOL.



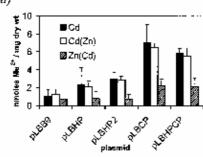


FIG. 5. Bioaccumulation of heavy metal ions by E. cott pope518 expressing Lamb-MESs in the double metal system. E. cott transformed with plasmids carrying specific insertions in Assas-133 (see Table 1) or the control plasmid pLEB9 was grown in MUS medium and induced at $OD_{200} = 0.3$ with IPTG. Cells were than grown until $OD_{200} = 0.4$ and supplemented with 13 μ M CelC2, and/or equimolar mixtures (15 μ M Concentrations of each metal ion) of either CelC2, and $COC2_2$ (a) or $COC2_3$ and $ZOC3_4$ (b). Their metal content was determined by stomic absorption spectroenersy after an additional 3.5 h of cultivation. The second metal or the mixture is indicated in brackets. The bass represent mean value of their indicated representations.

The absence of a contribution from the HP sequence for the Cu²⁺ binding was quite unexpected since the imidazotium group has been described as a ligand with a relatively high affinity for Cu²⁺ in biological systems (26). The competition of Cd²⁺ with Cu²⁺ or Zn²⁺ for LamB-MBSs in vivo was evaluated in order to elucidate this phenomenon. As shown in Fig. 5a, the amount of Cd²⁺ accumulated by cells expressing LamB-HP and LamB-HP2 from media containing an equimolar mixture of Cd²⁺ and Cu²⁺ (15 µM) caused less than a 10% decrease of bioaccumulation of Cd²⁺ by cells displaying HP sequences compared to the same cells grown in the presence of a single 15 µM Cd²⁺ (Fig. 5b). The cells expressing LamB-CP and LamB-HPCP accumulated a greater amount of heavy metals than the control from equimolar mixtures of both Cd²⁺ and Cu²⁺ (Fig. 5) However, the decrease in the amount of Cd²⁺ accumulated due to the presence of Cu²⁺ in the medium was significant (Fig. 5a). An apparently lower effect of Zn²⁺ on Cd²⁺ accumulation was observed with cells displaying the CP sequence (Fig. 5b).

DISCUSSION

The introduction of additional peptides serving as heavy metal ligands on the microbial surface represents one possible way for improving the metal binding properties of the biomass in terms of capacity, kinetics, and selectivity. The metal binding ability of the E coli cell wall has been studied in detail previously (2, 16, 23, 24). The specific native groups of the OM participating in metal binding are the polar head groups of phospholipids acting mainly at the inner layer of the OM and the acidic groups of the exposed (hydrophilic) polypeptides and at the outer half of the OM LPSs. The LPSs provide both carboxyl and phosphoryl groups as ligands. However, only the latter group is responsible for the high affinity of divalent metal ions for LPS (16). The peptidoglycan (PG) layer of E coli, which is most probably one molecule thick, binds metal ions via the carboxyl group of the n-glutamic acid of the peptide stem and the hydroxyl groups of the glycan backbone (23). The two-step deposition process may increase the apparent metal binding capacity of PG (3, 23) and would include the stoichiometric binding of metal ion, generating a nucleation site for the subsequent precipitation of metal above the stoichiometric amounts.

amounts.

The OM LamB protein of E. coli has been reported to tolerate the genetic insertions of heterologous peptides at positions between the structural codons 153 and 154 (5, 9, 11, 13, 22, 42, 43, 44). The genetic insertions of the HP and CP sequences into lamB-153 resulted in LamB-MBS hybrid proteins located in the OM. Furthermore, the LamB-MBSs retained their biological function as a lambda phage port, indicating a maintenance of their overall folding pattern. The Cd²⁺-to-peptide stoichiometry determined for synthetic HP and CP peptides is less than one metal ion per amino acid residue possessing metal binding properties (Fig. 1). This suggests specific folding of the sequence around the metal ion(s). The geometry of the MBSs of both HP and CP peptides is currently under study.

The increase of the metal binding capacity of the E. coli envelopes was significant (Table 1). Thus, the introduction of metal binding peptides onto the surface of the microorganism to be used as nonliving material for bioremediation may improve the opposes. It could be hypothesized that such an anonyme the opposes.

The increase of the metal binding capacity of the *E. coli* envelopes was significant (Table 1). Thus, the introduction of metal binding peptides onto the surface of the microorganism to be used as nonliving material for bioremediation may improve the process. It could be hypothesized that such an approach will improve not only the metal binding capacity of the biosorbent but also the kinetics of the process. An appropriate carrier for the surface display could be the only limiting factor. The C-terminal part of o-agglutinin could be such a carrier in yeast cells (39), which are being considered for use in the bioremediation of heavy metal ions (25).

We used *E. coli* as a model to evaluate changes of the metal

We used $E.\ coli$ as a model to evaluate changes of the metal binding properties due to surface display of the metal binding peptides. Surface display of HP and CP sequences resulted in a significant increase in Cd^{2+} bioaccumulation by growing $E.\ coli$ (Fig. 4 and 5). The number of Cd^{2+} binding sites generated by surface display of polyhistidine or metallothionein did not fully account for the amount of accumulated Cd^{2+} , as has been previously proposed (41, 42). The amount of LamB used in the expression system ranged from 1,000 to 5,000 molecules of protein per cell. This value is 2 to 3 orders of magnitude lower than the increment in Cd^{2+} accumulation due to the insertion of the MBS. It has been suggested that displayed peptides favor the interaction of Cd^{2+} with other bacterial structures by increasing the local concentration of metal ions (41, 42). The cell wall components involved could be, for example, LPSs ($E.\ coli$ pop6510 possesses the R type of LPS), which are known as compounds that directly interact with the LamB protein (36) and provide sufficiently effective metal ligands (16). The data

1096 KOTRBA ET AL. APPL. ENVIRON. MICROBIOL.

- KOTRBA ET AL.
 Hofbung, M. 1991. Expression of fixetign polypeptides at the Excherible coll cell surface. Methods Cell Biol. 4:77-105.
 Hoyla, B. D., and T. J. Bowaridga. 1984. Metal binding by the peptidoglycan saccutus of Excherible coll. 4:10-105.
 Hoyla, B. D., and T. J. Bowaridga. 1983. Binding of metallic tons to the cuter membrane of Excherible coll. Appl. Enrison. Microbiol. 36:204-211.
 Hoyla, B. D., and T. J. Bowaridga. 1983. Binding of metallic tons to the cuter membrane of Excherible coll. Appl. Enrison. Microbiol. 46:783-752.
 Bung, C. P., D. Westman, K. Quirk, and J. P. Hanag. 1988. The removal of cadmium (II) from dilute aqueous solutions by fungal absorbent. Water Sci. Technol. 20:369-376.
 Bubar, A. L., B. E. Helbells, and D. K. Kildy. 1990. Metal uptake by synthetic and biosynthetic Centrals, p. 249-291. In B. Volesky (ed.), Etcorption of heavy metals. CRC Press, Boox Ruton, Fla.
 Butchess, T. W., and T.-T. Ylp. 1902. Synthetic metal-binding protein surface domains for metal fon-dependent interaction chromatography. J. Chromatogr. 694:133-141.
 Kolda, T., D. Fester, B. Uoshitaka, and E. W. Davis. 1986. Amino acid sequence of human histidine-tich glycoprotein derived from the nucleotide sequence of its cDNA. Blochemistry 28:22220-2255.
 Kotrba, P., L. DoleBowt, M. Paville, and T. Ruml. 1996. Apid sercening of peptides for heavy metal binding Biotechnol. Technol. 18:773-778.
 Maccaskie, L. E., and A. C. R. Donn, A. K. Chastham, R. J. B. Jakaman, and A. J. Bloarmills. 1997. Codmium accumulation by Civobarte sp: the chemical statute of the accumulated metal precipitus and its Sociation on the bacterial cells. J. Gen. Microbiol. 133:539-544.
 Marvin, K. J. P., M. B. A. Ter Boss, and B. Wielbolt. 1989. Rulesse of outer membrane watcles from Ercherichic coll in self-generating Peccol gradients. Anal. Biochem. 18:647-51.
 Murgan, W. T. 1984. The h

- Riddles, P. W., R. L. Binkaley, and B. Zamor. 1983. Reassessment of Elimant's reagent. Methods Emyrand. 91:49-60.
 Sambrook, J., E. F. Fistock, and T. Maniatis. 1989. Molecular cloning: a laboratory manual, 2nd ed. Cold Speing Harbor Laboratory Press, Cold Speing Harbor Laboratory Press, Cold Speing Harbor Laboratory Press, Cold

- N.Y.
 Boum, C., A. Ceboin, and V. de Loronzo. 1996. Enhanced metallosception of bacterial cells displaying poly-His peptides. Nat. Eletechnol. 14:1017–1020.
 Soum, C., P. Kotrin, T. Rumi, A. Ceboin, and V. de Loronzo. 1998. Metal-losception by Encheckine coll cells displaying yeast and mammalism metal-lockiconins anchored to the outer membrane protein LamB. J. Bacteriol. 180:2280-2284.
- 43. Staidler, L., E. Romaut, and W. Flors. 1993. LamB as a carrier molecule for the functional exposition of IgG-binding domains of the Staphylococcus aureus protein A at the surface of Enchesichia coli K12. Mol. Gen. Genet. 236:187–192.
- 44. Su, G. T., E. Brahmbhastt, J. Wehland, M. Rohde, and K. N. Timmis. 1992. Construction of stable LamB-Shiga tonin B subunit hybrids: analysis of the expression in Subwards phylomiches co-e4 strains and stimmittion of B subunit-specific mucosal and seem antibody responses. Infect. Immun. 60: 3345-3359
- Sumaic man, S., and M. Hofmung. 1975. Maltone transport in *Eache-ichia* coli K-12: involvement of the bacteriophage lambda receptor. J. Bacteriol. 124:

- 112-118.

 46. Volusity, B., and Z. S. Holma. 1995. Biosorption of heavy metals. Biotechnol. Prog. 11:235-250.

 47. Watamaba, M. E. 1997. Phytoremediation on the brink of commercialization. Environ. Sci. Technol. 31:182-186.

 48. Yamamura, T., and T. Watamaths. 1993. Conformation analyses of (Cys-Pro-Leu-Cys)(210); the condel peptides for cystiene-containing metal bioding sites of metallo enzymes, p. 281-283. Ja N. Yamathara (ed.), Peptide chemistry. ESCOM, Leiden, The Netherlands.

 49. Yamamura, T., T. Sasaki, and M. Uski. 1993. Synthesis of the zinc complex of Boc-Ciu-Thr-Ile-Flis-Older the model peptides for the metal binding sites of fibonaciocide reductase, p. 284-286. Ja N. Yamathara (ed.), Peptide chemistry. ESCOM, Leiden, The Netherlands.

1979L0923 - EN - 23.12.1991 - 001.004 - 1

This document is meant purely as a documentation tool and the institutions do not assume any liability for its contents

►B

COUNCIL DIRECTIVE

of 30 October 1979

on the quality required of shellfish waters

(79/923/EEC)

(OJ L 281, 10.11.1979, p. 47)

Amended by:

Official Journal

No page date

►<u>M1</u> Council Directive 91/692/EEC, of 23 December 1991 L 377 48 31.12.1991

Corrected by:

▶<u>C1</u> Corrigendum, Of L 190, 12.7.2006, p. 99 (79/923/EEC)

₹B

COUNCIL DIRECTIVE

of 30 October 1979

on the quality required of shellfish waters

(79/923/EEC)

THE COUNCIL OF THE EUROPEAN COMMUNITIES,

Having regard to the Treaty establishing the European Economic Community, and in particular Articles 100 and 235 thereof,

Having regard to the proposal from the Commission (1).

Having regard to the opinion of the European Parliament (2),

Having regard to the opinion of the Economic and Social Committee (1),

Whereas the protection and improvement of the environment necessitate concrete measures to protect waters, including shellfish waters, against

Whereas it is necessary to safeguard certain shellfish populations from various harmful consequences, resulting from the discharge of pollutant substances into the sea;

Whereas the programmes of action of the European Communities on the environment of 1973 (*) and 1977 (*) provide that quality objectives are to be jointly drawn up fixing the various requirements which an environ-ment must meet, inter alia the definition of parameters for water, including shellfish waters;

Whereas differences between the provisions already in force or in preparation in the various Member States as regards the quality required of shellfish waters may create unequal conditions of competition and thus directly affect the functioning of the common market; whereas laws in this field should therefore be approximated as provided for by Article 100 of the Treaty:

Whereas it is necessary to couple this approximation of laws with Community action aiming to achieve, by means of wider-ranging provi-sions, one of the Community's objectives in the field of environmental protection and the improvement of the quality of life; whereas certain specific provisions must be laid down in this connection; whereas, since the specific powers of action required to this end have not been provided for in the Treaty, it is necessary to invoke Article 235 thereof:

Whereas, in order to attain the objectives of the Directive, the Member States will have to designate the waters to which it will apply and will have to set limit values corresponding to certain parameters, whereas the waters so designated will have to conform to these values within six

Whereas for the purpose of checking the quality required of shellfish waters, a minimum number of samples should be taken and the measurements relating to parameters set out in the Annex should be carried out; whereas such sampling may be reduced or discontinued in the light of the results of the measurements;

Whereas certain natural circumstances are beyond the control of the Member States and it is therefore necessary to provide for the possibility of derogating from this Directive in certain cases;

Whereas technical and scientific progress may make necessary the rapid adaptation of some of the requirements laid down in the Annexes; whereas, in order to facilitate the introduction of the measures required whereas, in order to isclinate the introduction of the measures required for this purpose, a procedure should be laid down establishing close cooperation between the Member States and the Commission; whereas such cooperation should take place in the Committee on Adaptation to

^(*) OJNo C 283, 30. 11. 1976, p. 3. (*) OJNo C 133, 6. 6. 1977, p. 48. (*) OJNo C 114, 11. 5. 1977, p. 29. (*) OJNo C 112, 20. 122. 1973, p. 3. (*) OJNo C 139, 13. 6. 1977, p. 3.

▼B

Technical and Scientific Progress set up by Article 13 of the Council Directive 78/659/EEC of 18 July 1978 on the quality of fresh waters needing protection or improvement in order to support fish life (1);

Whereas this Directive cannot, by itself, ensure protection of consumers of shellfish products; whereas proposals to this end should therefore be submitted by the Commission as soon as possible,

HAS ADOPTED THIS DIRECTIVE:

This Directive concerns the quality of shellfish waters and applies to this Directive concerns the quanty of sneathst waters and applies to those constal and brackish waters designated by the Member States as needing protection or improvement in order to support shellfish (bivalve and gasteropod molluses) life and growth and thus to contribute to the high quality of shellfish products directly edible by man.

Article 2

The parameters applicable to the waters designated by the Member States are listed in the Annex.

Article 3

- 1. Member States shall, for the designated waters, set values for the parameters listed in the Annex, in so far as values are given in column G or in column I. They shall comply with the comments contained in
- Member States shall not set values less stringent than those given in column I of the Annex and shall endeavour to observe the values in column G, while taking into account the principle set out in Article 8.
- 3. For discharges of effluents falling within parameters 'organohalo-genated substances' and 'metals', the emission standards laid down by the Member States pursuant to Council Directive 76/464/EBC of 4 May 1976 on pollution caused by certain dangerous substances discharged into the aquatic environment of the Community (2) shall be applied at the same time as the quality objectives and the other obligations arising from this Directive, in particular those relating to sampling.

Article 4

- Member States shall, initially within a two-year period following the notification of this Directive, designate shellfish waters.
- 2. Member States may subsequently make additional designations.
- Member States may revise the designation of certain waters owing in particular to factors unforeseen at the time of designation, taking into account the principle set out in Article 8.

Member States shall establish programmes in order to reduce pollution and to ensure that designated waters conform, within six years following designation in accordance with Article 4, to both the values set by the Member States in accordance with Article 3 and the comments contained in columns G and I of the Annex.

Article 6

For the purposes of implementing Article 5, the designated waters shall be deemed to conform to the provisions of this Directive if samples of such waters, taken at the minimum frequency specified in the Annex, at the same sampling point and over a period of 12 months, show that

⁽¹⁾ OJ No L 222, 14. 8. 1978, p. 1. (2) OJ No L 129, 18. 5. 1976, p. 23.

▼B

they conform to both the values set by the Member States in accordance with Article 3 and the comments contained in columns G and I of the Annex, as regards:

- 100 % of the samples for the parameters 'organohalogenated substances' and 'metals':
- 95 % of the samples for the parameters 'salinity' and 'dissolved oxygen':
- 75 % of the samples for the other parameters listed in the Annex.

If, in accordance with Article 7 (2), the sampling frequency for all the parameters in the Annex except 'organohalogenated substances' and 'metals' is lower than that indicated in the Annex, the abovementioned values and comments shall be complied with in the case of all the samples.

Instances in which the values set by Member States in accordance with Article 3 or the comments contained in columns G and I of the Annex are not respected shall not be taken into consideration in the calculation of the percentages provided for in paragraph 1 when it is the result of a disaster.

Article 7

- The competent authorities in the Member States shall carry out sampling operations, the minimum frequency of which is laid down in the Annex.
- 2. Where the competent authority records that the quality of designated waters is appreciably higher than that which would result from the application of the values set in accordance with Article 3 and the comments contained in columns G and I of the Annex, the frequency of the sampling may be reduced. Where there is no pollution and no risk of deterioration in the quality of the waters, the competent authority concerned may decide that no sampling is necessary.
- If sampling shows that a value set in accordance with Article 3 or a comment contained in columns G or I of the Annex is not respected, the competent authority shall establish whether this is the result of chance, a natural phenomenon or pollution and shall adopt appropriate measures.
- 4. The exact sampling point, the distance from this point to the nearest point where pollutants are discharged and the depth at which the samples are to be taken shall be fixed by the competent authority of each Member State on the basis of local environmental conditions in particular.
- The reference methods of analysis to be used for calculating the value of the parameters concerned are set out in the Annex. Laboratories which employ other methods shall ensure that the results obtained are equivalent or comparable to those specified in the Annex.

Article 8

Implementation of the measures taken pursuant to this Directive may on no account lead, either directly or indirectly, to increased pollution of coastal and brackish waters.

Article 9

Member States may at any time set more stringent values for designated waters than those laid down in this Directive. They may also lay down provisions relating to other parameters than those provided for in this Directive.

Article 10

Where a Member State considers designating shellfish waters in the immediate vicinity of a frontier with another Member State, these States shall consult each other in order to determine the stretches of such waters to which this Directive might apply and the consequences to be

▼B

drawn from the common quality objectives; these consequences shall be determined, after formal consultations, by each State concerned. The Commission may participate in these deliberations.

Article II

The Member States may derogate from this Directive in the event of exceptional weather or geographical conditions.

Article 12

Such amendments as are necessary for adapting to technical and scientific progress the G values for the parameters and the methods of analysis contained in the Annex shall be adopted by the Committee set up by Article 13 of Directive 78/659/EBC in accordance with the procedure laid down in Article 14 thereof.

Article 13

For the purpose of applying this Directive, Member States shall provide the Commission with information concerning:

- the waters designated in accordance with Article 4 (1) and (2), in summary form,
- the revision of the designation of certain waters in accordance with Article 4 (3),
- the provisions laid down in order to establish new parameters in accordance with Article 9.

Where a Member State applies the provisions of Article 11, it shall forthwith notify the Commission thereof, stating its reasons and the periods anticipated.

More generally, Member States shall provide the Commission, on a reasoned request from the latter, with any information necessary for the application of this Directive.

Article 14

▼<u>M1</u>

At intervals of three years the Member States shall send information to the Commission on the implementation of this Directive, in the form of a sectoral report which shall also cover other pertinent Community Directives. This report shall be drawn up on the basis of a questionnaire or outline drafted by the Commission in accordance with the procedure laid down in Article 6 of Directive 91/692/EEC ('). The questionaire or outline shall be sent to the Member States six months before the start of the period covered by the report. The report shall be sent to the Commission within nine months of the end of the three-year period covered by it.

The first report shall cover the period from 1993 to 1995 inclusive.

The Commission shall publish a Community report on the implementation of the Directive within nine months of receiving the reports from the Member States

▼B

Article 15

- Member States shall bring into force the laws, regulations and administrative provisions necessary to comply with this Directive within two years of its notification. They shall forthwith inform the Commission thereof.
- Member States shall communicate to the Commission the texts of the main provisions of national law which they adopt in the field governed by this Directive.

(1) OJ No L 377, 31. 12. 1991, p. 48.

 $1979 L0923 - EN - 23.12.1991 - \ 001.004 - 6$

▼<u>B</u>

Article 16

This Directive is addressed to the Member States.

ANNEX

QUALITY OF SHELLFISH WATERS

	Parameter	G	1	Reference methods of analysis	Minimum sampling and measuring frequency
1.	pH pH unit		7—9	Electrometry Measured in situ at the time of sampling	Quarterly
2.	Temperature ⁶ C	A discharge affecting shell- fish waters must not cause the temperature of the waters to exceed by more than 2 °C the temperature of waters not so affected		Themometry Measured in situ at the time of sampling	Quarterly
3.	Coloration (after filtration) mg Pt/l		▶ C1 A discharge affecting shellfish waters must not cause the colour of the waters after filtration to deviate by more than 100 mg Pt/I from the colour of waters not so affected ◀	— Filter through a 0.45 μm membrane Photometric method, using the platinum/ cobalt scale	Quarterly
4.	Suspended solids mg/l		A discharge affecting shellfish waters must not cause the suspended solid content of the waters to exceed by more than 30 % the content of waters not so affected	 Filtration through a 0.45 µm membrane, drying at 105 °C and weighing Centrifuging (for at least five minutes, with mean acceleration 2.800 to 3.200 g), drying at 105 °C and weighing 	Quanterly
5.	Salinity %	12 to 38 %e	= 40 % Discharge affecting shellfish waters must not cause their allinity to exceed by more than 10% the salinity of waters not so affected.	Conductimetry	Monthly
6.	Dissolved oxygen Saturation %	≥ 80 %	> 70 % (average value) Should an individual measurement indicate a value lower than 70 %, measurements shall be repeated An individual measurement may not indicate a value of less than 60 % unless there are no harmful consequences for the development of shellfish colonies	Winkler's method Electrochemical method	Monthly, with a minimum of one sample representative of low oxygen conditions on the day of sampling. However, where major daily variations are suspected, a minimum of two samples in one day shall be taken

▼<u>B</u>

	Paramotor	G	1	Reference methods of analysis	Minimum sampling and measuring frequency
7.	Petroleum Is ydrocarbons		Hydrocarbons must not be present in the shell-fish water in such quantities as to: — produce a visible film on the surface of the water and/or a deposit on the shellfish, — have hammful effects on the shellfish	Visual examination	Quarterly
8.	Organohal ogenated su balances	The concentration of each substance in shell fish flesh must be so limited that it contributes, in accordance with Article 1, to the high quality of shellfish products	The concentration of each substance in the shell-fish water or in shellfish flesh must not reach or exceed a level which has harmful effects on the shellfish and larvae	Gas chromatograpny after extraction with suitable solvents and purification	Half-yearly
9.	Metals Silver Ag Arsenic As Cadmium Cd Chromium Cr Copper Ca Mercuny Hg Nickel Ni Lead Pb Zinc Zn mgt	The concentration of each substance in shell find flesh must be so limited that it contributes in accordance with Article 1, to the high quality of shellfish products	The concentration of each substance in the shell-fish water or in the shell-fish baster or method from the same of exceed a level which gives rise to learnful effects on the shell-fish and their larvee The specific effects of these metals must be taken into consideration.	Spectrometry of atomic absorption preceded, where appropriate, by concentration and/or extraction.	Half-yearly
10.	Faccal coli forms/100 ml	≤ 300 in the shell sish flesh and intervalvalar liquid (*)		Method of dilution with fermentation in liquid substrates in at least three tubes in three dilutions. Subculturing of the positive tubes on a confirmation medium. Count according to MPN (most publishe number). Incubation temperature 44 4 \times ± 0 \times	Quarterly

1979L0923 — EN — 23.12.1991 — 001.004 — 8

	Parameter	G	1	Reference methods of analysis	Minimum sampling and measuring frequency
	Substances affecting the taste of the shellfish			Examination of the shellfish by tasting where the presence of one of these substances is presumed	
12.	Saxitoxin (produced by dinoflagel- lates)				

Abbreviations: G = gside

(1) However, pending the adoption of a Directive on the protection of consumers of shellfish products, it is essential that this value be observed in waters in which live shellfish directly edible by man.

1979L0923 — EN — 23.12.1991 — 001.004 —

References:

IWA Publishing, *Basic Water Treatment* (3rd Edition), Chris Binnie, Martin Kimber and George Smethurst.

Porteous, A. (2000) *Dictionary of Environmental Science and Technology*, 3rd edition, London, John Wiley and Sons.

Open University (2006), *T308 Block, Potable Water Treatment*, Milton Keynes, The Open University.

Publish America Baltimore (2007), Heavy Metals, Art Smith

The Royal Society of Chemistry (1990), *Pollution, Causes Effects and Controls*, (2nd Edition), Roy M Harrison

The Royal Society of Chemistry (2001), 16 Issues in Environmental Science and Technology, Assessment and Reclamation of Contaminated Land, R. E. Hester and R. M. Harrison

Pergamon Press Ltd (1973), Progress in Water Technology, Volume 3, (collection of authors – consultation papers)