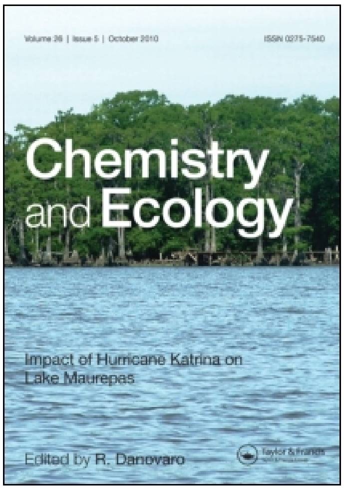

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COLONIZATION AND SELECTION OF FLY-ASH SUBSTRATES BY MARINE INVERTEBRATES

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Fly-ash dumped on the sea bed causes a local impoverishment of the benthos. Experiments on ash in sea water have shown bacterial colonization to occur, at a slower rate than on natural silt, limited meiofaunal colonization after 18 months, selection by meroplanktonic larvae for the ash, though inhibition of development after settlement, and selection against ash by mobile benthic adults. The major inhibition to colonization is the lack of organic content in the ash.

KEY WORDS Fly-ash, marine invertebrates, colonization

INTRODUCTION

The burning of pulverized coal in coal-fired power stations produces two forms of ash, of which the fine powder known as pulverized fuel ash (pfa) or fly-ash contributes some 70% of the total. A 2000 MWe power station can produce some 1×10^6 tonnes of fly-ash per year. While a significant proportion of this ash finds commercial outlets, for example building block manufacture or as a cement admixture, the remainder (some 60% in the U.K.) has to be disposed of economically.

Since the late 1950s, the Blyth and Stella Power Stations, on the north-east coast of England, have been dumping fly-ash on to the local designated spoil ground in the North Sea, under licence from the Ministry of Agriculture, Fisheries and Food (MAFF). Field surveys of the sea bed in this area (Bamber, 1984; 1989a) showed a localised impoverishment of the benthic fauna at the vicinity of the dump site, attributed to a smothering analogous to excessive deposition, with a significant reduction in the benthos over some 13 km^2.

Fly-ash is chemically comparable to natural sedimentary material of a fine silt particle size, with no organic content, and with pozzolanic properties leading to reduced porosity and permeability (Bamber, 1980). Circumstantial evidence suggests that it is not toxic to marine invertebrates (Bamber, 1989b). After cessation of dumping, the redevelopment of a benthic infaunal community at a dump site will depend on recolonization by mobile adults and settlement by planktonic larvae of benthic species.

A series of laboratory experiments has been undertaken to examine four aspects of recolonization processes, using the benthic species from the Blyth area. The fly-ash used in the experiments was supplied from Blyth Power Station by the Central Electricity Generating Board. Natural sedimentary material was obtained from the ash-free sublittoral sea bed off Northumberland (assumed to be representative of sediment found originally in the vicinity of the dump site); this material was sterilized at 60°C for 24 hr, then sieved through a 500 μm mesh with fresh water, and the fine fraction retained, being dried and re-sieved to produce "natural silts" as required.

BACTERIAL COLONIZATON OF FLY-ASH BEARING SUBSTRATES

Fly ash is initially inorganic. Many studies have demonstrated the importance of the presence of organic matter in the sediment to successful colonization by marine benthic organisms (see Bamber, 1978, p. 75 for a review). Bacteria play the major role in adsorptive and developmental organic enrichment of sediments (e.g. Zobell, 1942; Paerl, 1974). The rate of development of a bacterial epiflora on fly-ash was therefore examined as one basis for estimating the time scale for recovery of ash-bearing sea bed substrates.

Methods

Bacterial development on four experimental sediments was analysed by measuring the increase in organic nitrogen in the sediments over a period of five weeks (see Newell, 1965, for the contribution of bacterial population growth to the organic nitrogen content of a substrate). The experimental sediments were: 1) pure fly-ash, 2) natural silt (dried, sieved through a $\leq 44\,\mu$m mesh, then maintained in sterile sea-water for two weeks), 3) a 1 : 1 mixture of these two, and 4) burnt natural silt (natural $\leq 44\,\mu$m silt, burnt in a furnace at 600°C until completely oxidized, then maintained in sterile sea-water for two weeks).

For each sediment type, samples were retained for analysis at time zero, and aliquots were placed in ten watch-glasses. The 40 samples were arranged at random on a perforated perspex base connected to an air supply, which allowed uniform aeration of the water around the watch-glasses without any disturbance (or intermixing) of the sediments. The whole was immersed in 7 litres of sea water which had been filtered (1.2 μm ultrafine glass fibre) to remove silt and organic particles. To ensure a demonstrable bacterial flora, the water was innoculated with 20 ml of unfiltered sea water which had been overlying a stable sea bed sample from the Northumberland offshore, maintained in the laboratory for 1 week.

At weekly intervals, two watch-glasses for each sediment type were removed and dried at 55°C for 24 hours. Two subsamples from each watchglass were analysed for organic nitrogen by the micro-kjeldahl technique using the pyrazolone method for analysis, as described by Martin (1968). The lack of distillation procedures and the replacement of titration by spectrophotometry simplifies the analysis and removes some of the main areas of possible experimental error. Since the analysis was intended only to compare ash-bearing and natural sediments, the results were expressed in equivalent percentages of calibration solution (3.57×10^{-5} M ammonium chloride, equivalent to $5 \times 10^{-4}\,\mathrm{g\,l^{-1}}$ N).

Results

All four sediments tested showed development of a bacterial flora, as represented by organic nitrogen (Figure 1). The greatest development occurred in natural silt,

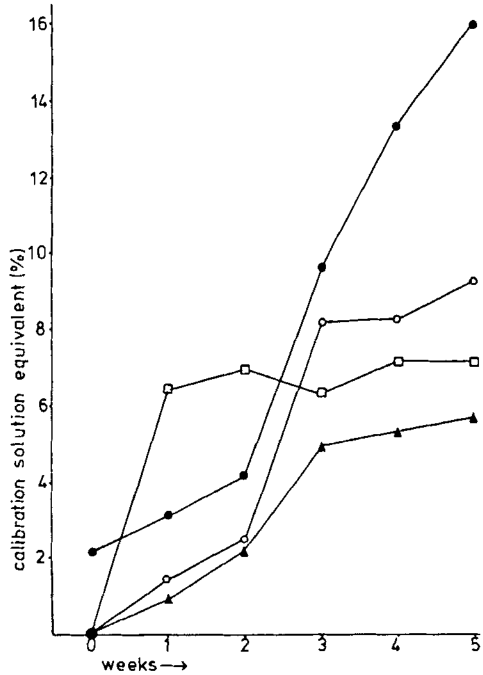

Figure 1 Development of organic nitrogen content over five weeks in four experimental sediments: natural sterilized ≤44 μm silt (●), pure pfa (▲), a 1 : 1 mixture of these two (○) and burnt ≤44 μm silt (□).

achieving a level after 5 weeks twice as high as any other test sediment. The pure ash consistently showed the lowest rate of development, while the 1 : 1 mixture was intermediate between these two. After a rapid initial rise, the burnt silt nitrogen content levelled out to a content intermediate between the mixed sediment and the pure ash.

It has been shown that fly-ash exhibits pozzolanic aggregation over this time scale (Bamber, 1983), which activity would reduce the particle surface area available for bacterial exploitation: such a process may account for the lower organic nitrogen levels in the ash compared to the burnt silt.

Clearly, fly-ash in sea water does support the development of a bacterial flora, and will thus show a gradual increase in organic content (and therefore suitability for faunal exploitation) over time, but at a lower rate than normal sedimentary material.

COLONIZATION OF PURE ASH IN A CONTINUOUS FLOW OF SEA WATER

Faunistic colonization of pure ash in the field will occur in response to a complexity of factors (for example, seasonality, larval availability, water quality, leachates from or adsorption onto the ash). Controlled monitoring of a sample of ash in the field is subject to difficulties such as human interference. Therefore an ash sample was exposed in the laboratory to a flow of natural sea water for an extended period, in order to observe 'natural' colonization.

Methods

A plastic tray of area 2800 cm^2 was filled to a depth of 8 cm with fly-ash. The tray was established in a constant through-flow of sea water supplied from an intake just below extreme low water of spring tides (ELWS) off a sandy beach. The water contained suspended particulate matter as well as larvae from the nearshore plankton. The tray was left in the flowing sea water for 18 months. At the end of this time the ash had a 1 cm covering layer of natural silt.

The ash was analysed for benthic fauna and for physical sedimentary characteristics. Identical analyses were undertaken on two trays of $\leq 500\,\mu m$ natural Northumberland sea bed silt which had been established in the aquarium some months previously; though not valid as "experimental controls", these data offer valuable comparative information on the conditions and colonization of the "normal" sea bed sediment. Samples for physical and biological analyses were taken with cores (3.2 cm diameter), sectioned (where appropriate) at 1 cm depth intervals. These were analysed for water content (weight loss at 100 °C), Eh (using a platinum wire electrode; see Bamber, 1984), and a measure of organic carbon content (% weight loss at 600°C, with correction for the content of inorganic carbon based on analysis, by the same method, of fresh fly-ash and natural silt retained prior to establishment of the experimental trays).

Results

The most obvious epifaunal colonizers of the ash tray were the two ascidians *Ciona intestinalis* and *Ascidiella aspersa*; specimens of both were attached at the ash surface by an elaborate ramification of stolons, in marked contrast to the simpler base exhibited by these species when attached to normal hard substrates. No ascidians had colonized the trays of natural silt. Silt, contributed in large part by their own egestion, had accumulated around the bases of the ascidians, and it constituted a microhabitat distinct from the ash itself.

Infauna of the ash tray was analysed from ten 8 cm depth cores. Away from the ascidian stolons, these cores yielded in total one specimen of each of *Abra nitida, Corophium insidiosum* and *Polydora ciliata,* and three unidentified oligochaetes. The comparative natural silt trays supported large numbers of five species of polychaete, seven species of mollusc and two species of amphipod, demonstrating their availability from the plankton in the water flowing over the ash.

Meiofauna was collected from three separate 8 cm depth core samples, sectioned at 1 cm intervals and extracted by the suspension sieving method of Warwick and Buchanan (1970), using a 90 μm mesh sieve. The 1 cm silt layer over the ash supported a low density meiofauna (compared to the natural silt). This density rapidly decreased with depth, and no meiofauna was found below 3 cm (i.e. 2 cm into the ash). The meiofauna in natural silt was present to a depth of 4.5 cm (Figure 2). Some 75% of the meiofaunal community was nematodes, about 20% foraminiferans, and the remainder harpacticoid copepods; with the low densities involved, no notable differences in meiofaunal community composition were evident.

Organic carbon was present in the superficial layer of silt overlying the ash, but not in the ash itself (Figure 2). This is reflected in virtually constant Eh readings down into the ash, with no redox potential discontinuity (RPD). With no

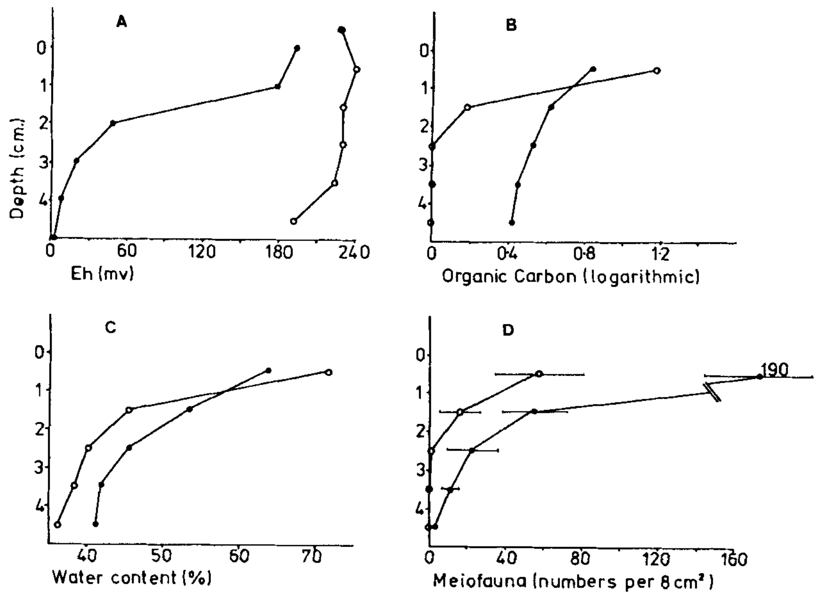

Figure 2 Vertical distribution of sediment parameters in 18 month pfa culture (open circles) and comparative natural mud sediment (filled circles): A, Eh (mv); B, Organic carbon (log % weight loss at 600°C); C, Water content, % (porosity); D, total meiofaunal numbers (per 8 cm^2).

detected organic carbon (or fauna) below 2 cm, there is no material available to be reduced, so the Eh remains "high". Water content (≡porosity) was consistently lower in the ash than in comparable natural mud (see also Bamber, 1980).

SETTLEMENT OF PLANKTONIC LARVAE

Meroplanktonic larvae of benthic species can show a degree of substrate selectivity when settling. Laboratory experiments were undertaken offering planktonic larvae the choice of a range of substrates including various ash concentrations.

Methods

Larvae of spionid polychaetes (*Malacocerus tetracerus* and *Polydora ciliata*), gastropod molluscs (unidentified veligers) and lamellibranch bivalves (a mixture of *Fabulina fabula, Hiatella arctica, Pododesmus squamula, Mysella bidentata, Venus striatula* (? = *Chamelea gallina*), *Spisula subtruncata, Donax vittatus* and *Mytilus edulis*) were collected from the inshore Northumberland plankton using a 115 μm mesh net. Experimental sediments were made up and established in petri dishes (for polychaete larvae) or ice-cube trays (for mollusc larvae), and allowed to stabilize in trays of filtered sea water.

The polychaete larvae were given a simple choice between all combinations (duplicated) of two of the following substrates: 1) ≤500 μm natural silt ("normal mud"); 2) ≤44 μm natural silt; 3) ≤44 μm burnt natural silt (see bacterial colonization experiment above); 4) pure fly-ash; 5) sea-water-matured fly-ash (from the 18 month colonization tray described above). Mollusc larvae were given a multiple choice between six substrates, being the five as used for the polychaetes plus a 1 : 1 mixture of pure fly-ash and ≤500 μm sieved mud, as well as empty "control" compartments. For each experiment, care was taken to ensure that all substrates presented the same surface area for settlement. There were 4 replicates of each substrate.

Aliquots of the planktonic larval cultures were introduced to the "choice trays" which were then maintained for seven days in the dark. After this time the water, together with any remaining plankton, was siphoned off. Each substrate sample was washed through an 88 μm mesh sieve, and the residue examined for settled larvae.

Results

Table 1 shows the preferences expressed by the spionid larvae: the substrate of the test pair which contained the larger number of larvae was considered to be preferred where the difference was significant ($p < 0.05$). The categories of the rows (A) of Table 1 are scored for preference against the column categories (B), scoring 1 if A is preferred, ½ if no preference is shown, and 0 if B is preferred. Summing the horizontal rows gives a ranking of preference of the five substrates.

The rankings surprisingly demonstrate that the normal mud was the least preferred substrate. Close analysis of each selection suggests that the larvae show an initial preference for the substrate with the finer particle size: normal mud was the coarsest of the sediments tested. However, when the two substrates had similar particle sizes, preference was consistently shown for the substrate with the higher organic content. Thus, while pure fly-ash is preferred to normal mud, owing to particle size selection, ≤44 μm sieved mud is preferred to fly-ash, and indeed to any other test substrate.

The settled larvae were also examined for their metamorphosis to the benthic form; metamorphosis occurred only in substrates with some organic content (including sea-water-matured fly-ash: Table 1). Thus, for example, while settlement selection between fly-ash and normal mud showed a preference for the ash

Table 1 Substrate settlement preferences shown by spionid larvae, with percentage metamorphosis to benthic form.

A ↓ B→	*Pure pfa*	*Normal mud*	*≤44 μm mud*	*Burnt mud*	*Sea water matured pfa*	*Total*
Pure pfa	—	1	0	½	0	1½
Normal mud	0	—	0	0	½	½
≤44 μm mud	1	1	—	1	1	4
Burnt mud	½	1	0	—	½	2
Sea water matured pfa	1	½	0	½	—	2
% Metamorphosis	0	95	90	0	35	

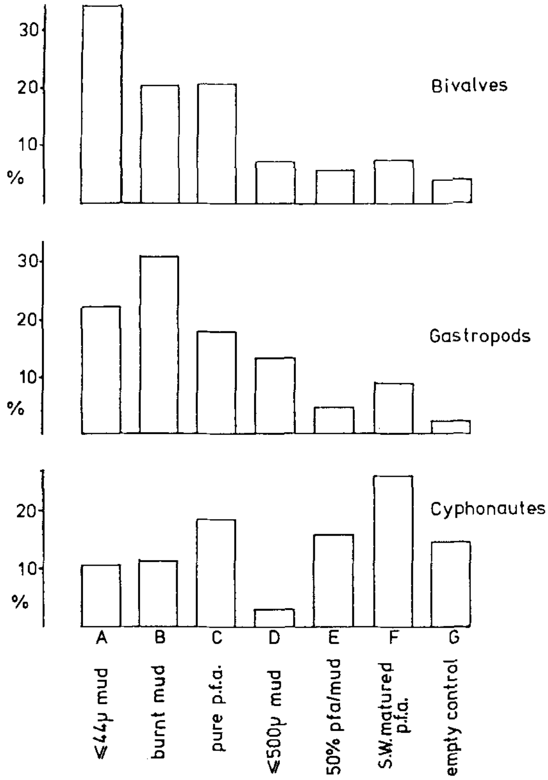

Figure 3 Percentage settlement distribution after seven days of molluscan and bryozoan larvae given a choice of seven substrates.

of the order of 50 : 1, 95% of larvae settling on the mud metamorphosed within one week, whereas none metamorphosed on the fly-ash.

Figure 3 shows the percentages of mollusc larvae settling in the offered substrates. Both groups showed a preference for finer particle size: 75% of the bivalves and 71% of the gastropods settled in ≤44 μm particle-size substrates. Within the particle-size groups, the larvae demonstrated some preference for organic matter: bivalve larvae showed equivalent settlement on burnt mud and pure fly-ash, but 68% higher settlement on unburnt ≤44 μm mud. Survival of settled bivalves was lower in ash-bearing than in non-ash-bearing substrates.

Bryozoan cyphonautes larvae were also present in the mollusc larvae cultures. These are animals which would be expected to prefer "coarser" substrates, and 10% more cyphonautes larvae settled in total in the coarser substrate group (D + E + F, Figure 3) than in the finer (≤44 μm) group (A + B + C). Within these groups, they showed a preference for ash-bearing substrates, possibly attributable to pozzolanic activity increasing the "particle-size" of the substrate. Settlement in the empty "control" chamber was higher than in any of the non-ash-bearing substrates.

If these results are translated to the sea bed situation, then larvae from the plankton would readily settle on fly-ash sediments, but further development would be inhibited, at least until the ash had had time to develop an organic component via bacterial colonization (presumably assisted by any previous larvae which had settled and died in the sediment). The fauna most able to develop initially on such substrates, when dumping has ceased, would be more likely to consist of epifaunal forms such as the bryozoans.

SUBSTRATE SELECTION BY BENTHIC ADULTS

To determine further indications of the recolonization of dumped fly-ash, experiments were conducted to test the responses of a variety of benthic macrofaunal species with mobile adults, when given a choice between ash-bearing and normal sediments.

Methods

Experimental sediments were prepared by mixing known proportions (by volume) of fly-ash and ≤500 μm natural silt, and introduced into the four corners of choice trays bearing temporary partitions. The trays were carefully flooded with sea water and left to stabilize for 24 hours: the partitions were then carefully removed, avoiding cross contamination of the sediments, to give a continuous substrate in the tray of three or four different mixes. The seven species and the substrates tested are shown in Table 2. Each species was tested separately, except for the two spionid polychaetes which were tested together.

Animals were introduced by placing equal numbers in each sector, then maintained in darkness. The animals were observed at regular time intervals until distribution between the different sediments was relatively constant, or, in the case of species not readily counted from the sediment surface (e.g. infaunal polychaetes), the animals were extracted completely from each sector at the end of the experiment (48 hours or longer) and counted.

Table 2 Results of sediment choice experiments for 7 species of benthos in ash-bearing and normal sediments (*SW = fly-ash matured in sea water): the sediments are ranked in order of selected preference (most preferred first; = denotes no preference). The animals were either counted at intervals, or after a longer elapsed time, as shown in the third column. The fourth column shows the numbers of individuals tested per replicate × the number of test replicates.

Species ↓	*% pfa in substrates tested, in order of selected preference*	*Time scale*	*No.*
Ampelisca brevicornis	0, *SW** = 100	intervals	21 × 4
Diastylis rathkei	0, 50, *SW** = 100	intervals	20 × 4
Eudorella truncatula	0, 30, 60, 100	intervals	20 × 3
Echinocardium cordatum	0, 50, *SW** = 100	intervals	10 × 4
Nephtys hombergi	0, 25, 50, 100	48 hours	12 × 4
Malacocerus tetracerus	0, 30, 60, 100	1 week	≃17 × 4
Polydora ciliata	0, 30, 60, 100	1 week	≃20 × 4

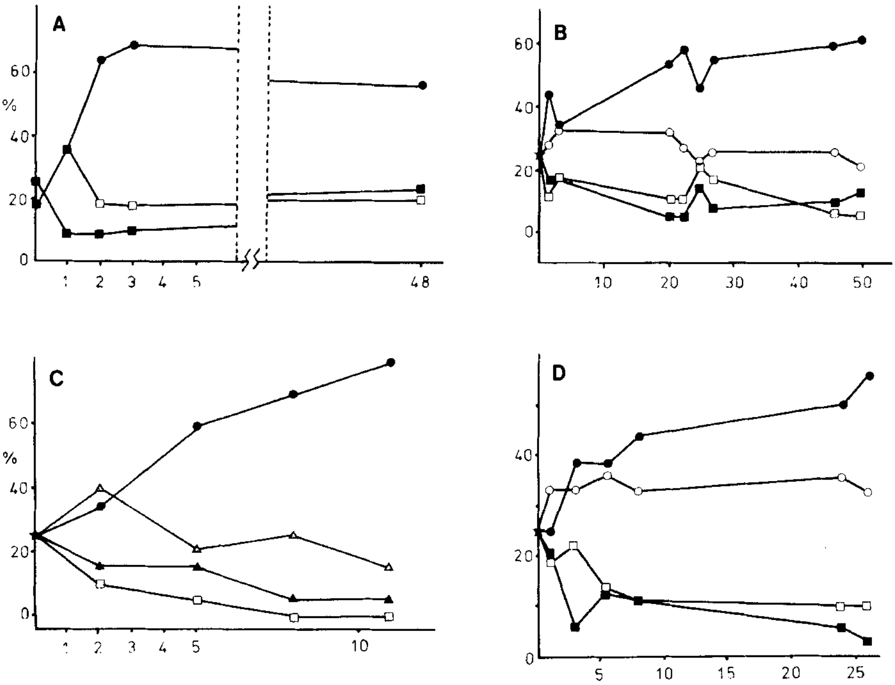

Figure 4 Percentage distribution of mobile adult benthic invertebrates in a choice tray over time (hours), given a choice of different fly ash concentrations in natural sediment (●—0% ash; △—30%; ○—50%; ▲—60%; □—100%; ■—sea-water-matured ash): A, *Ampelisca brevicornis*; B, *Diastylis rathkei*; C, *Eudorella truncatula*; D, *Echinocardium cordatum*.

Results

The amphipod *Ampelisca brevicornis* rapidly showed a preference for normal mud as opposed to fly-ash (X^2, $p < 0.05$ after 3 hours), while showing no significant preference between fresh or sea-water-matured ash (Figure 4A). The cumaceans *Diastylis rathkei* and *Eudorella truncatula* showed a rapid order of preference between the four substrates offered in their experiments, both preferring lower concentrations of ash in the sediment (Figure 4B and C). *D. rathkei* did not distinguish between pure and sea-water-matured ash; *E. truncatula* showed complete avoidance of pure ash within 11 hours, at which time 80% of animals were in normal mud (benthic cumaceans are known to show orthokinetic activity resulting in congregation in favourable substrates: see Wieser, 1956).

The heart urchin *Echinocardium cordatum* gave similar results to those of *D. rathkei*, with significant selection against ash after 8 hours (Figure 4D). The comparatively larger numbers of *E. cordatum* remaining in the 50% ash in mud substrate probably reflected the larger ambit of this species, and thus the potential for "crowding" in the pure mud compartment. When individual *E. cordatum* were introduced to the choice trays at the interface between two test substrates, their initial movement to burrowing showed a significant selection for lower fly-ash concentrations.

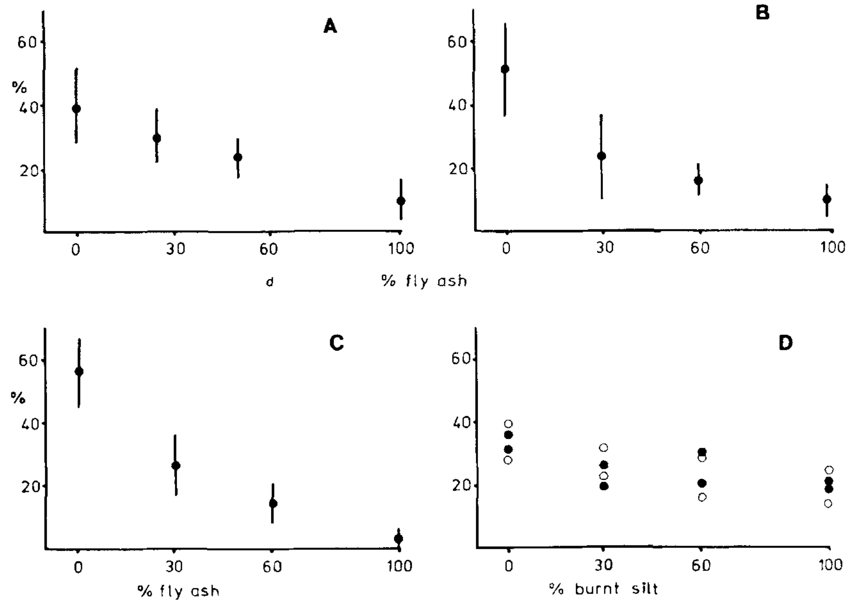

Figure 5 A to C: percentage distributions in offered sediments of polychaetes given a choice of four concentrations of fly-ash (means with ranges from four replicates): A, *Nephtys hombergi* after 48 hours; B, *Malacocerus tetracerus* after 1 week; C, *Polydora ciliata* after 1 week. D, distribution of *M. tetracerus* (●) and *P. ciliata* (○) after 1 week given a choice of four concentrations of ≤44 μm burnt natural silt (data values from duplicate experiments.

The polychaetes tested were counted at the ends of the experiments, *Nephtys hombergi,* counted after 48 hours, showed a consistent significant preference for lower fly-ash content (Figure 5A). The spionids *Malacocerus tetracerus* and *Polydora ciliata* were counted after one week. The distributions of both showed a significant negative correlation to fly-ash content (Figures 5B and C).

The results from all the experiments are identical, in that all the species of adult macroinfauna tested showed a significant preference for substrates of lower fly-ash content, the animals distributing themselves in the experimental sediments in an inverse relationship to the ash concentrations. Absolute selection for the most favoured substrate was probably inhibited by ambit size requirements, since the animals were at an artificially high density in the experiments.

That those animals with the choice showed no distinction between fresh and sea-water-matured fly-ash suggests that selection is based not solely on organic content. Further tests done in duplicate on the spionid polychaetes using burnt ≤44 μm mud in place of fly-ash showed a similar though less marked, selection for less "contaminated" sediments (Figure 5D). The substrate selections would appear to be based upon criteria of both organic content and particle size as affected by pozzolanic activity of the ash.

DISCUSSION

The poor colonization seen in the tray of pure ash maintained in sea water for 18 months was confirmed by the planktonic larval experiments, which showed that epifaunal species are most likely, at least initially, to develop successfully on ash-bearing substrates. The low organic content of the ash inhibits development of infaunal larvae, despite their preferred selection for its particle size. The mobile adults of the benthic macroinfauna also prefer a lower ash content, possibly because of organic content (the species tested were all deposit feeders). Thus the recolonization of ash-bearing sediments will be inhibited owing to these animals showing a tendency to move off or avoid such substrates in favour of "non-polluted" sediments. Overall, the results suggest that the major inhibition to colonization is the lack of organic content in the ash, a feature that will improve as bacterial colonization progresses. The time scale of the latter is hard to predict: in the tests described here, a bacterial flora did develop, but at a slow rate compared with normal sedimentary material. After 18 months in the continuous flow tray the material was still comparatively unattractive to meiofauna. Nevertheless, it is worth noting that there is some survival of planktonic larvae and some macrofaunal adult colonization at most levels of ash content.

In the dynamic situation of the natural sea bed, mixing of the ash with both settled and depositing natural sedimentary material is inevitable: all the experiments have indicated that increasing natural mud content in the fly-ash is proportionately preferable to both faunistic and bacterial colonization. Indeed, the most recent field sampling of the ash dump off Northumberland has shown a marked improvement in the benthic fauna, compared to 13 years previously, at individual sampling sites assumed to have experienced no recent dumping (Bamber, 1989a). In the interim there had been an approximately 50% reduction in the quantity of ash dumped per annum.

In the long term, with a non-toxic material such as fly-ash, the progression of bacterial colonization, adsorption, and meiofaunal and macrofaunal colonization should eventually lead to an apparently normal benthos. A field survey of an historic dump site would be invaluable in confirming this.

SUMMARY AND CONCLUSIONS

Dumping of fly-ash on the sea bed can cause local elimination of the benthic fauna due to smothering by the excess artificial "deposition". The redevelopment of a benthic fauna is dependent for recruitment on colonization by both larvae and adults. Four aspects of fly-ash colonization have been investigated in the laboratory.

Bacterial development on ash-bearing sediments was monitored via the increase in organic nitrogen in sediments over five weeks. Colonization of ash occurred at a significantly lower rate than that for natural sediment, and was slightly lower than the rate for incinerated natural silt.

The maintenance of pure fly-ash in a flow of sea water for 18 months demonstrated that colonization from pelagic settlement was restricted to a few epifaunal species, and limited to the surface. Although infaunal species occurred in superficial sedimentary silt (the development of which was enhanced by egested material from the epifauna), these infaunal species showed no tendency to burrow into the ash itself after 18 months.

When given the choice of natural and ash-bearing sediments, planktonic larvae of infaunal species often showed an initial settlement on the ash. However, subsequent development of the larvae was inhibited in the ash substrate. It is postulated that initial selection may be based on particle size, with secondary selection for organic content. The low organic content of fly-ash may inhibit subsequent development of settled larvae.

In adult sediment choice experiments, macroinfaunal selection, for all species (including echinoderms, peracarids, molluscs and polychaetes), showed a significant inverse relationship to ash content, attributed to criteria of particle size and organic content.

Overall, the results indicate that the major inhibition to colonization is the lack of organic content in the ash, a feature that improves as bacterial colonization progresses.

Acknowledgements

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