FLUORESCENCE MICROSCOPIC OBSERVATION OF BEACH SANDS AND GRAVELS CONTAMINATED BY C – TYPED HEAVY OIL AND REMEDIATION METHODS

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ABSTRACT

Was the removal method right thing to do at that place when the heavy oil spill accident happened in the Hokuriku district ? We developed a remediation method using warmed – up sea water as an environmental treatment of beach sands and gravels polluted by C – typed heavy oil. The method is safe, simple, easy, and low cost. Fluorescence microscopic observation also revealed the effect of removed heavy oil. Additionally, we observed emulsified C – typed heavy oil associated with bacteria under epifluorescence microscope as on – going natural recovery interactions.

INTRODUCTION

Recently, remediation trials at the environments contaminated by crude or heavy oil have been fast getting attentions in the world. Since the *Exxon Valdez*'s accident resulted in spilling abundant crude oil in March, 1989, various environmental removal methods have been considered. However, in this *Nakhodka*'s incident, most of cleaning tasks of C – typed heavy oil spread all over the Hokuriku shoreline demanded man – power by local people and volunteers. The coastline of Hokuriku was so complicated. Especially in winter the weather of Hokuriku area was so severe that people could not remove the heavy oil by hands overall. That meant we needed an appropriate method for each site. The government's attitude toward this incident made many people frustrated because they seemed to take action only for the moment. For instance, in Shioya and Katano seashores, Kaga City in Ishikawa Prefecture, the government ended up dumping oily sands as a result of their short – term decisions just to collect drifted heavy oil and sands together by bulldozers.

We investigated all over the Hokuriku seashore after heavy oil reached the area, studied the real situation of C – typed heavy oil contamination and the characteristic of emulsified oil, and then approached a method of separating heavy oil and sands, which would not damage ecosystem, after discussing treatments of sands contaminated by C – typed heavy oil. We have developed a safe, simple, and easy method with low cost. We also show some fluorescence microscopic photographs, such as various bacteria existing around heavy oil and emulsified C – typed heavy oil degradation by ultraviolet – ray under the microscopy.

COLLECTING HEAVY OIL ON THE SITE AND THE ISSUES

Commercial C – typed heavy oil contains approximately 97 % of hydrocarbon with a little N and S. The viscosity ranges from less than 180 to 3000 mm^2/s in 50 ℃ (Hanashima, 1995a, b). The C – typed heavy oil spilled out from a tanker gets enough sea water to turn into emulsified condition in one to two days. The color of heavy oil is originally black, but it will be changed to chocolate and then brown within one to two days, depending on seawater concentrations. The color of heavy oil drifted in Anto, Mikuni Town of Fukui Prefecture showed black on January 9th, 1997, while the next day on January 10th, it changed into chocolate color in mousse – like state. The mousse – like heavy oil was not lipophilic enough to be absorbed into cellulose pads or neither was mixed in a neutralizer. When the common commercial C – typed heavy oil whose specific gravity is 0.93 to 0.97 is emulsified, and sea water contents become more than 80 %, the specific gravity tends to be near 1.0 and the viscosity also increases. Moreover, if the heavy oil adheres to sands or dead microorganisms, the apparent specific gravity of heavy oil can be more than that of seawater (approximately 1.02 to 1.03), and it could happen that those heavy oil chunks will sink down to the bottom of ocean as oil balls (Higashi *et al.*, 1976). Although the spilled oil is gradually diffused and degraded, it definitely affects many oceanic organisms (Nelson – Smith, 1972). Emulsified C – typed heavy oil had quite high water contents to be hardly burnt in usual methods on the scene. When the viscosity is high, it becomes more difficult to suck by vacuum machine.

We did not have any effective ways to remove emulsified C – typed heavy oil at steep mountain coast in the Hokuriku area. Therefore, the only solution to collect heavy oil was literally “man – power”, which people used their hands occasionally with dippers. Especially, in sandy beaches, such as Shioya and Katano seashores in Kaga City, local people and volunteers tried to do their best to separate heavy oil from polluted sands using handy sieves. On the other hand, in graveled beaches, such as Anto in Mikuni Town and Atake seashore in Wajima City, people even used chopsticks to pick up gum – like heavy oil or wiped each gravel with cloth. In rocky seashores, some places were washed off by high temperature and pressure nozzle water with detergents. These methods not only were impractical or ineffective but also left great concerns to the environmental ecosystem. Particularly, in Shioya and Katano seashores, as a result of using shovel cars, a huge amount of C – typed heavy oil had just been abandoned outside for a few months. Afterwards, some part of it was burnt as garbage, another part was just buried in the same seashore, and the other was buried in industrial waste plants with cements. In the waste plant, to prevent from leaking carcinogenic substances in C – typed heavy oil, they would have to watch and manage it forever. In the seashore where they buried heavy oil, we had to concern underground water contamination by toxins. Also, taken those examples of oil spill accidents in the world, using abundant detergents and high pressure hot water pumping would be the least effective for oceanic and seashore ecosystems or would delay the recovery in the contaminated area (Shimizu *et al.*, 1972).

SEPARATION METHODS OF SANDS AND GRAVELS FROM HEAVY OIL

In Shioya and Katano seashores, a big issue was taken up in the air before the huge mounts of sands polluted by C – typed heavy oil: What would we do with these?

Therefore, we carried out an experiment which could resolve the problem. Sands contaminated by C – typed heavy oil were dipped into a beaker of warmed seawater, and mixed well. The sands were separated instantly from heavy oil: the cleaned sand grains were sunk, whereas the oil rose to the surface, and seawater stayed in the middle. We also applied to gravels polluted by heavy oil at Mikuni seashore. Approximately 100 g of sands contaminated by C – typed heavy oil collected in Shioya seashore was in a beaker filled with approximately 800 ml of seawater warmed in about 40 ℃ (Fig. 1A). Immediately after the sands added into the beaker, the heavy oil started getting off from the sands, and after a few minutes, the oil was completely separated from sands as shown in Fig. 1B. The C – typed heavy oil (O) was lighter than the seawater (W), while the sands (S) sank in the bottom of beaker. After the sands and the heavy oil were completely separated, the heater was taken away and the sands were observed closely (Fig. 1C). The heavy oil was washed off from sands after treatment (a) to compare with sands before treatment (b) in spite of a little oily smell. We also tried to clean up oily gravels with warmed seawater for a few minutes. In case of low specific gravity of emulsified C – typed heavy oil, adding salt would help to make the cleaning task because the specific gravity of seawater gets higher.

FLUORESCENCE MICROSCOPIC OBSERVATIONS OF EMULSIFIED C – TYPED HEAVY OIL, BEACH SANDS, AND BACTERIA

Epifluorescence microscope enables us to observe only substances which are excited by dropping necessary waves, extracting light waves through a filter from Hg ramp. PAHs like C – typed heavy oil are excited by ultraviolet – ray and emit fluorescence, so that we can observe emulsified C – typed heavy oil very well although it is not clearly visible under general optical microscopes. Moreover, dyeing nucleic acids, such as DNA and RNA in microorganisms, can make photographs of micro organisms visibly clearer. Here in this study, fixed samples were stained with DAPI for three minutes to observe under epifluorescence microscope (Nikon EFD – 3; Digital camera: Nikon COOLPIXE 995). For epifluorescence microscopy, a filter (UV – 1A wavelength of exposed light: 365 mm) was used for DAPI – staining observation. Color films of ISO 400 and 800 were available for micrographs, and exposure time of each picture was one to five seconds.

Fig. 2A and B show micrographs of beach sands contaminated by C – typed heavy oil collected in Shioya seashore. Under transmitted visible rays, Figure 2A shows that colored minerals (b) and C – typed heavy oil in sands (em) stayed close each other. Emulsified C – typed heavy oil emitting fluorescence clarified that C – typed heavy oil was also attached on the surface of colored minerals (Fig. 2B). The emulsified C – typed heavy oil was observed to be sparkled and spread right away in the arrow of Fig. 2B. According to Shimizu (1978), C – typed heavy oil oxidized chemically by ultraviolet – ray is degraded into alcohols, aldehydes, ketones, and organic acids. Fig. 2C and D show cleaned sand granules after separation from heavy oil in the beaker with warmed sea water. Under transmitted visible – rays, two colored granules were not contaminated by emulsified C – typed heavy oil (C), while under ultraviolet – ray (D), fluorescence of heavy oil was slightly detected on the left granule. Additionally, the right sand granule emitted a little blue fluorescence as a result of self – fluorescence. As shown in the above, comparing A and B to C and D, fluorescence microscopic observation determined a state of sands separated from heavy oil, suggesting it was pretty efficient to wash sand using by buoyancy method of warmed sea water.

Figure 3 shows a series of micrographs of a process which the emulsified C – typed heavy oil (Fig. 2B arrow) was sparkled by ultraviolet – ray. It took only a few seconds or more that about 100 μm heavy oil was spread. Bigger oil drop was degraded from the rim into oil slick in a few minutes.

Fluorescence micrograph shows bacterial colony (B) growing along emulsified C – typed heavy oil (O) collected in Nagahashi seashore in Suzu City (Fig. 4). Due to the microscopic observation of bacteria, DNA was dyed by DAPI stain. The DAPI fixed sample produces blue light if excited by 365 μm wave light, while DAPI itself or combined by other substances look yellow if >390 μm light (Porter and Feig, 1980). C – typed heavy oil included 50 % of seawater was absolutely emulsified in the DAPI stain sample and surrounded by bacilli in about 5 μm and cocci in 1 μm under transmitted optical microscope. However, we could see few bacteria in sea water which did not have emulsified heavy oil. With UV – 1A filter, the DAPI stain sample shows C – typed heavy oil (O) in self – fluorescent yellow and the complex of DNA and DNA of bacillus or coccus typed bacteria (B) in blue fluorescent light (Fig. 4). Bacteria emitting blue fluorescent inhabited in cleavages of emulsified heavy oil.

CONCLUSION

The C – typed heavy oil tragedy happened along our beautiful Hokuriku seashores as a result of the *Nakhodka* accident in January, 1997. Tremendous efforts by local people and volunteers made it possible to collect much of spilled heavy oil. On the other hand, in sandy seashores like Shioya in Kaga City, heavy oil was collected by shovel cars. The huge amount of sands contaminated by C – typed heavy oil was dumped in waste areas in the end. Now, we have to concern about second disasters like underground water pollution or soil pollution after they buried the heavy oil in the ground. Also, it is very important to build oil fences or put absorption mattress along the place where sands or rocks are washed by emulsifiers to prevent ecosystems from damaging, which did not happen enough in this incident.

In this study, for the purpose of separating beach sands and gravels from C – typed heavy oil, we have shown a useful way of warmed seawater buoyancy. We also have observed the process of C – typed heavy oil by ultraviolet – ray and how local bacteria live with heavy oil in the collected sample. In addition, after field investigations and laboratory experiments, we actually started some field trials to show the method for local people (Fig. 5). A couple of trials resulted in a nice, little device that helped separating sands and pebbles from heavy oil. Despite the difficulty in getting high temperature of seawater in an oil drum, considering air – temperature was so low due to the strong north wind, we designed a cleaning system after gathering drifted wood for fuel at the beach, as shown in Figure 6. Using an oil drum and steel baskets made repeatedly cleaning available in seawater about 40 ℃ on beach. You could add oily sands and pebbles in the basket, rinse and then put back cleaned – up pebbles, sands and seawater to the nature. This method is simple, easy, environmentally safe and cost – effective, so that it should be applied to an environmental remediation system.

In the *Exxon Valdez*'s case in the USA, people tried to learn from the incident and had completed an environmental recovery manual in five years. The area is still under observation by ecological scientists and other specialists. In Japan, although we had the same kind of incidents, like the *Juliana* caused the crude oil spill off shore of Niigata City in 1971 (Honma and Kitami, 1974; Aoki *et al.*, 1975), and Mitsubishi had C – typed heavy oil spill in Mizushima in 1974, in our country few people seemed to have learned from those lessons to handle the *Nakhodka* incident this time, nor have bothered to face it for seven years. Now, we have to say aloud that we have gotten to learn from this incident. It is time to carry on follow – up environmental investigations for middle and long term, which will not let us excuse no matter what specialty we have. That will be the only way to leave for the future. We will also have to start editing a manual of oil spill incidents, bioremediation techniques, and remediation methods of oil at polluted area.

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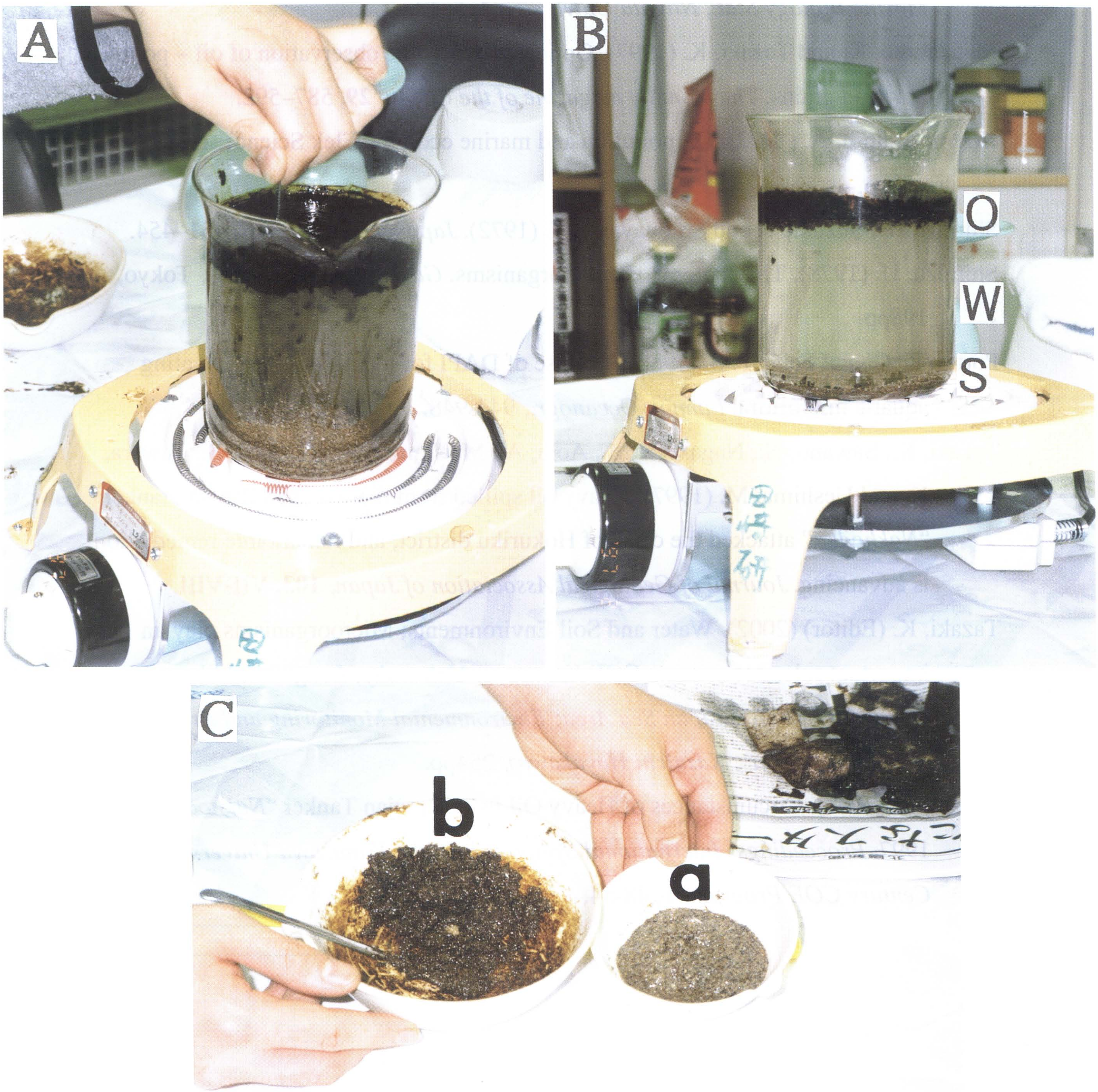

Figure 1 Separation experiments of C – typed heavy oil from sand grains by warmed seawater. Warming up seawater, adding polluted sands and mixing (A). Immediately separated into heavy oil (O), seawater (W), and sands (S) in the beaker (B). Comparing sands before treatment (b) with after treatment (a).

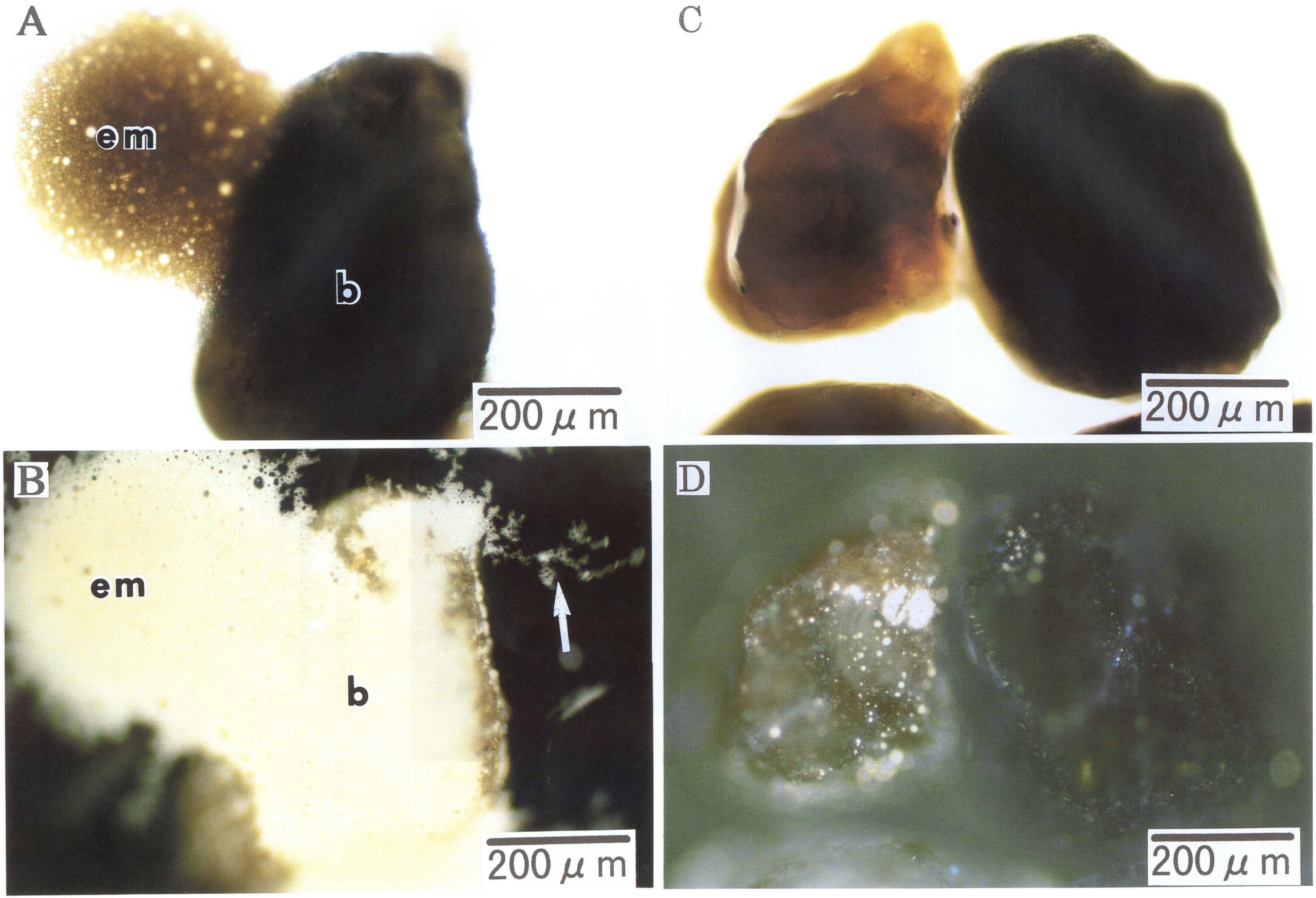

Figure 2 Optical micrographs of polluted beach sand granules. A; sands before treatment under transmitted visible – rays, B; under ultraviolet – ray, showing yellowish emulsified oil. C; after – treatment sands under transmitted visible – rays, D; under ultraviolet – ray after treatment, em; emulsified oil, b; sand granule.

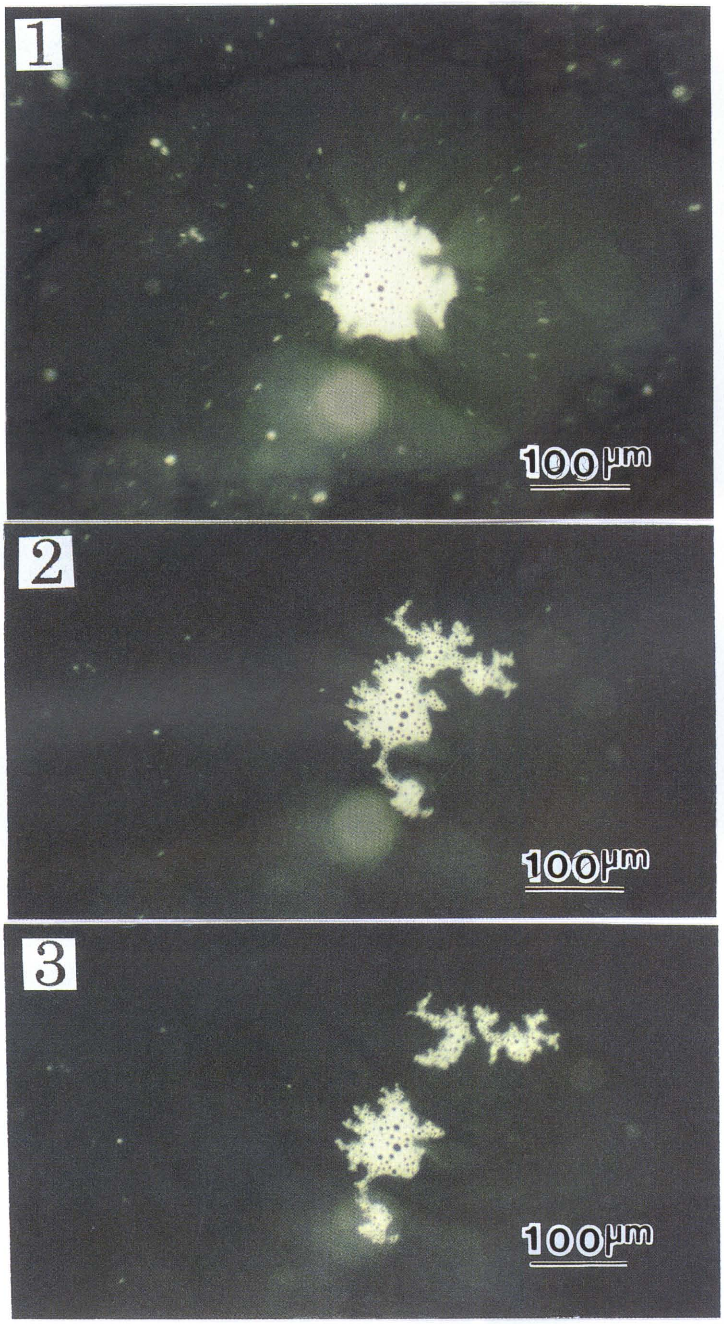

Figure 3 Process of C – typed heavy oil degradation by ultraviolet – ray. Only a few seconds to be sparkled from 1 to 3.

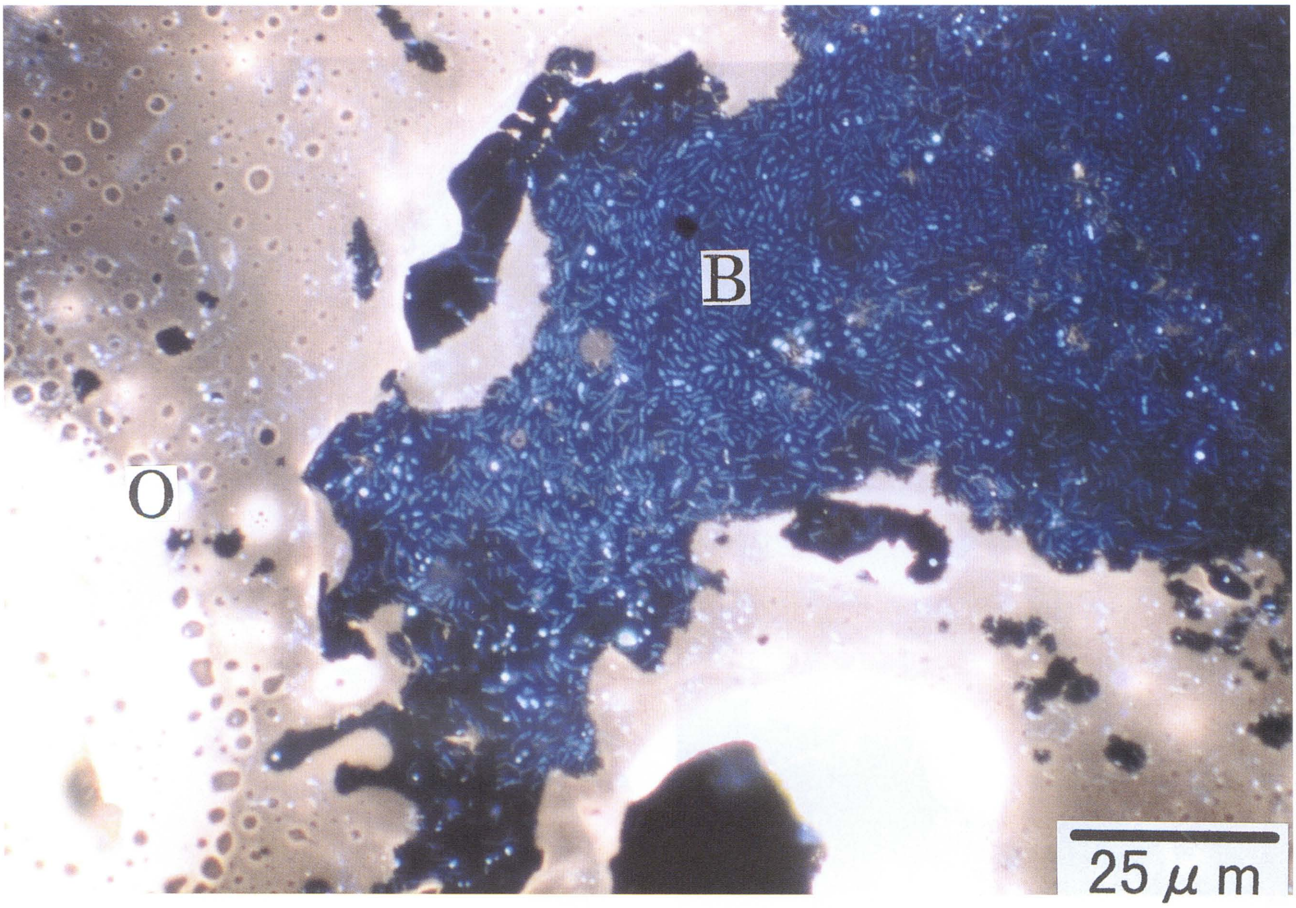

Figure 4 Fluorescence microscopic photograph of yellowish C – typed heavy oil (O) and blue bacterial colony (B) in and around emulsified heavy oil.

Figure 5 Remediation tasks of sands or gravels from heavy oil on the site. Warming up seawater in about 40 °C, adding contaminated sands or gravels in the drum, then getting the oil float to the surface.

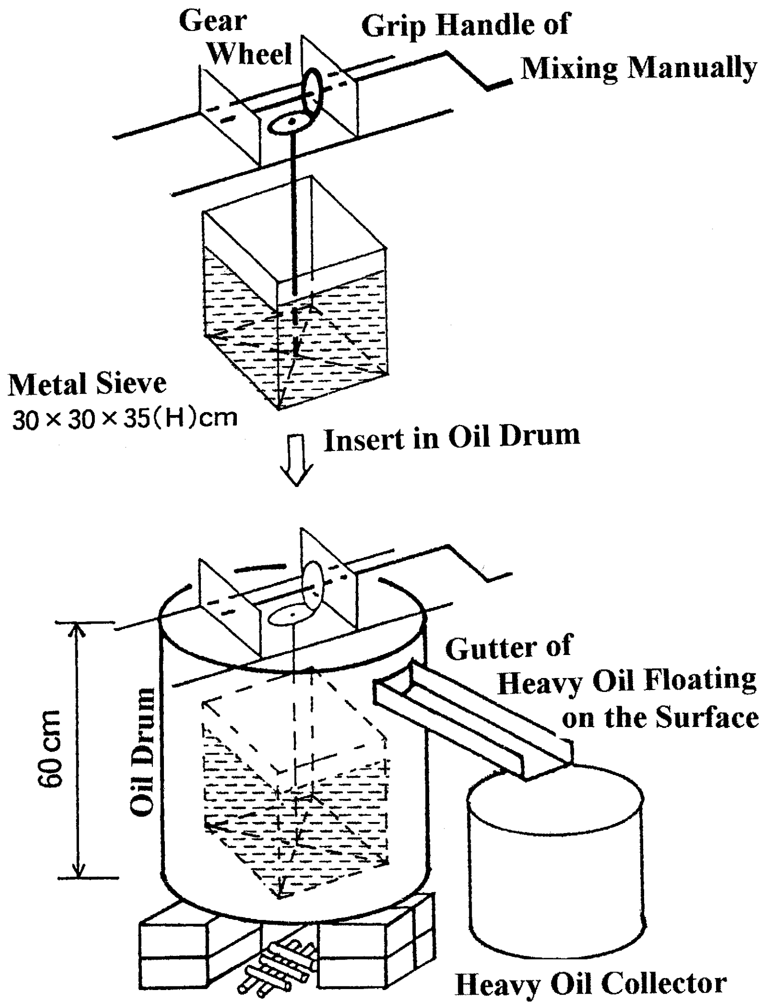

Figure 6 Schematic image of the device system for cleaning sands and pebbles contaminated by heavy oil.