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SAND TREATMENT PROCEDURES FOR CHEMICAL ANALYSIS AND BEACH SAND QUALITY

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Abstract

Sand contamination is an important factor when assessing the health hazard potential of public beaches. The goals of this study are to assess the performance of four sand washing procedures on the removal of surface adsorbed chemical species, and to identify which chemical components are best suited for monitoring the health quality of beach sand. Four washing treatments were investigated: contact, stirring, sonication, and boiling. Sand samples were collected from three Portuguese beaches and the wash water analysed. The observed variables were specified in two main groups: 1) physicochemical parameters comprising sulphates, pH, and conductivity; and 2) pollution indicators including nitrogen and phosphorous chemistries, chemical oxygen demand (COD), and oxidability. The Group I measurements did not exhibit significant differences with sand treatment ($p=0.89$), but there were differences in the Group II measurements due to the higher COD and oxidability values ($p<0.01$). The best extraction yield was obtained with sonication. In terms of a suitable health quality marker, neither group exhibited significant variation with beach location or sampling period of year. Individually, however, several chemical parameters were found to be significant on the basis of sampling period ($p < 0.01$) and beach location ($p < 0.001$). The COD and oxidability values were also found to vary significantly on the basis of sampling period ($p < 0.01$ and $p< 0.001$, respectively). COD was determined to the best sand quality indicator.

Keywords: beach sand, chemical indicators, chemical analysis, COD, sand treatment

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1. Introduction

Beaches represent the unconsolidated sediment that lies at the junction between water (oceans, lakes and rivers) and land and are usually composed of sand, mud or pebbles (WHO, 2003).

It is a popular holiday destination, mainly for families with children, not only due to the pleasure of swimming but also for leisure and sports activities on the sand and consequently a significant percentage of time is spent on the beach sand itself rather than in the water. Therefore, it is important to monitor the quality of the beaches' sand.

The beach's environmental quality can play an important role in its competitiveness. In fact, the beaches environmental quality has become an important decision criterion for tourists when choosing their holiday destination, for environmental and public health reasons (Geldenhuys and Merwe, 2014; McKenna et al., 2011).

Sand beaches can become a repository of accumulated pollution if contaminated by garbage, animal waste, or pathogenic microorganisms and parasites that are washed ashore (Halliday and Gast, 2011; Heaney at al., 2009; Sabino et al., 2011; Sato et al., 2005; Whitman et al., 2006). These substances or

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pathogens can afflict swimmers, kayakers, or surfers when they ingest the contaminated water or sand. Children are most at risk because they may put contaminated sand or water in their mouth. People exposed to these chemical or microbiological contaminants can contract numerous illnesses including gastroenteritis, hepatitis A, ear and nose infections, skin diseases and rashes (Colford et al., 2007; Heaney at al., 2009; Kay et al., 1994; Marion et al., 2010; Paraskaki et al., 1994; Sanchez-Nazario et al., 2014). Although these diseases rarely pose life-threatening risks, they do cause severe discomfort and can result in substantial economic losses to society due to lost work days. The health risks are more severe for children, the elderly, pregnant women, and for those with a compromised immune system (Boehm et al., 2009; GLIN, 2014).

With regard to information available to the public, only Directive 2006/7/EC (EC Directive, 2006) and the "Blue Flag of Europe" award (FEE, 2011) provide indicators for the environmental quality of beaches. However, these quality indicators are only related to the seawater quality. There is no communitarian or national legislation regarding beach sand quality and therefore, there isn't any direct information on that subject to the population. However, the quality of the beaches itself can not be exclusively related to water quality but must also consider the sand's quality. For this reason, sand monitoring has often been recommended and applied by researchers (Heaney at al., 2009; Olanczuk-Neyman and Jankowska, 2001; Pereira et al., 2013; Sabino et al., 2011, 2014).

The composition of sand is highly variable, depending on the local rock sources and conditions. Although clean, washed beach sand is primarily quartz, which is relatively inert, but can contain other mineral nutrients, or other inorganic compounds. Some of these inorganic compounds are contaminants, such us phosphorus, and nitrogen compounds (ammonium, nitrates, and nitrites). The organic matter is also a sand contaminant. These substances can act as a culture medium for the development of several microorganisms (Sabino et al., 2014; WHO, 2003).

Microorganisms are a significant component of beach sand. Sands and sediments provide habitat in coastal zones, where fecal bacteria populations may persist, and in some cases grow (Sabino et al., 2011, 2014; Whitman et al., 2014). Bacteria, fungi, parasites and virus have all been isolated from beach sand, and a number of genera and species encountered in sand are potential pathogens, although transmission by this route has not been demonstrated in epidemiological studies (Heaney et al., 2009; Olanczuk-Neyman and Jankowska, 2001; Pereira et al., 2013; Sabino et al., 2014; WHO, 2009; Yamahara et al., 2012). It is not frequent, the monitoring of specific pathogens on beach sand and sediment, although there is evidence of its presence in these habitats (Halliday and Gast, 2011). In the beach monitoring programs, the common procedure to evaluate the microbiological water quality and protect the beachgoers health at recreational beaches is to test the water for bacterial indicators of fecal pollution. Although, the beaches whose water quality exceed the standards are subsequently closed, there are not any evaluation of sanitary quality of sands. Furthermore, the World Health Organization has expressed concern regarding the beaches sand quality as these may constitute a reservoir of infectious agents, especially in beach zones where the use of the sand is greater (WHO, 2003).

Various factors that promote the survival and dispersion of faecal microorganisms and pathogens in beach sands have been proposed. These include the nature of the beach, tidal phenomena, sewage outlets, the season, the presence of animals and the number of bathers (Mendes et al., 1998; Papadakis et al., 1997; WHO, 2003). The majority of these factors, can increase the amount of organic matter, and therefore increase the further development of microorganisms because organic matter provides nutrients and habitat to microorganisms living in the sand.

The decomposition of organic matter by microbial activity release several inorganic compounds to sands, such us, nitrates, ammonia, and phosphates (McLachlan and Brown, 2006). Therefore, these inorganic compounds can be characteristics of some sands, and their variation depends among other variables, on organic matter input. Therefore, the quality of beach sand cannot be limited to its microbiological evaluation but must also consider its chemical quality. Moreover, results regarding chemical quality can be used as indicators of sand's microbiological quality as the increase of some organic chemical parameters, is most of the times related to a microbiological development (Bertron, 2014). It is necessary to define which are the most representative chemical parameters for the assessment of a sand's quality, and which of these parameters could be used as sand pollution chemical indicators (chemical tracer) (Halliday and Gast, 2011; WHO, 2003).

To date there are no data related to the chemical quality of beach sands because all studies are solely focused on the assessment of the microbiological quality of the sands (ICREW, 2007), and for microbiological analysis, the aim of sand extraction procedures were the recovery of any microorganism from sand, which is improved with high ratio sand: water. (INSA, 2001; 2003). Many factors affect the results of sand's chemical analysis. These factors range from the most basic principles of laboratory operation (*e.g.* equipment cleanliness, quality control) to temperature, ratio sand:water, shaking speed, and contact time between the water and the sand. However, the sand washing procedure should be the most representative due to their influence in the recovery of the chemical substances adsorbed in the sand's grains (Hoskins and Ross, 2011).

There are different methods of extraction of inorganic compounds from sands, and some of these methods are separate extraction methods dedicated to the extraction of a specific inorganic compound. For example, the most common analytical approach to determine inorganic N and P concentrations are potassium chloride extraction and bicarbonate extraction, respectively (Hoskins and Ross, 2011). Another methods, such us, ion exchange membrane technique (IEM) was also developed as an alternative to chemical extraction methods to measure these inorganic substances (Barghouthi et al., 2012). However, a good extraction procedure aims to improve the efficiency of both inorganic and organic matter, with a lowest cost with respect to time, but also to a less laborious process. Therefore, the extraction procedures by means of a single extractant was tested using four sand washing procedures with water.

Some chemical substances classified as tracers can be used to evaluate potential sources of contaminants collected from different hydrologic settings. The global evaluation of results from all individual tracers is intended to confirm, refine, or refute interpretations based on the individual information by each tracer (Izbicki et al., 2009).

Portugal is one of those destinations in Europe that is well known for its good beaches. The Portuguese Coastal area is divided into five regions (north, center, Lisbon and Tagus valley, Alentejo and Algarve) and the most frequented beaches are those located in the southern area of the Tagus River. For this reason, we selected for this study the most representative Portuguese beaches, such us Costa da Caparica's beaches, beaches on the Alentejo coastal area and beaches in Vilamoura (Algarve). The Costa da Caparica is situated near Lisbon and for this reason their beaches are usually very crowded during summer time. The Alentejo is the region located just to the north of Algarve. The beaches of Alentejo face the open Atlantic to the west and are almost as extensive as those on the southern coastal area of Portugal, but are considerably less developed and thus are less crowded. Vilamoura city (south of Portugal) is one of the most attractive tourist destinations in Portugal and it hosts hundreds of thousands of visitors annually.

This study was designed to choose the most effective sand washing procedure for the characterization of beach sand. This sand washing procedure was then applied to sand samples from several of Portugal's tourist beaches. By sampling over time in addition, this study allowed us to understand the temporal variation of several chemical indicators of pollution. Considering both spatial and temporal variations, a preferred quality indicator is sought.

2. Material and methods

2.1. Chemicals and instrumentation

All the aqueous solutions and serial dilutions were prepared by dilution with ultra-pure water (Mili Q System, Millipore, Billerica, MA, USA). Ultra-pure water was produced on a Milli Q Gradient A10 system, from Millipore (Molsheim, France). For filtration purposes, a gravimetric analysis filter, 0.45 µm, mixed cellulose esters, with thin pad 37 mm, white from Millipore (Molsheim, France) was used.

The samples sonication was made with an ultra sound bath (Selecta, Barcelona, Spain), and the stirring and heating of aqueous solutions were performed using a hot/stirring plate, Bibby Sterilin CD 162 (Staffordshire, UK) with magnetic stirring bars 25 mm × 8 mm from Azlon (Bibby Sterilin, Staffordshire, UK). The conductivity was determined with a CyberScan CON 200, supplied by Eutech Instruments (Thermo Fisher Scientific Inc.), and the calibration and control of the equipment was made with several conductivity standards (84, 147, 1413 and 12880 µS cm^{-1}, 25ºC) supplied by Reagecon (Shannon, Co. Clare, Ireland). The pH was measured using a pH meter, Crison, model micropH 2002 (Barcelona, Spain) with a glass electrode (Crison, Barcelona, Spain). Its calibration and control were made with pH standards (pH 4, pH 7, pH 6 and pH 9) supplied by Crison (Barcelona, Spain).

The digestion of samples for determination of chemical oxygen demand was made on COD reactor, model 45600, Hach (Loveland, USA) with COD Digestion Vials, Low Range, 40 mg L^{-1} were supplied by Hach (Loveland, USA). Potassium permanganate titrant 0.01M and oxalic acid titrant 0.01M were supplied by Carlo Erba (Milan, Italy).

The absorption spectrophotometry was performed using a double beam UV/Visible spectrophotometer, HITACHI U-2000 (Tokyo, Japan). The sodium nitrate, sodium nitrite, sodium sulphate, potassium phosphate monobasic, ammonium chloride, potassium hydrogen phthalate and resorcinol (*pro-analysis* grade) were supplied by Merck (Darmstadt, Germany).

2.2. Validation studies

The linear range of the spectrophotometric analysis was assessed by analysing several solutions containing the standards in different concentration levels. The study of linearity included the R square evaluation and the statistical linearity test from ISO 8466-1 (ISO, 1990), for determining the test value PG required for the F-test. If PG ≤ F, the non-linear calibration function does not lead to a significantly better adjustment: the calibration function is linear.

The values of limit of detection (LOD) and limit of quantification (LOQ) were calculated using Eq. (1) and Eq. (2), respectively.

$$LOD = \frac{\left[3.3\, S_{y/x}\right]}{b} \tag{1}$$

$$LOQ = \frac{\left[10\, S_{y/x}\right]}{b} \tag{2}$$

where: $S_{y/x}$ is the residual standard error and b the slope of the calibration curve.

The repeatability of each method was studied after analysis of ten replicate samples of spiked reagent water. The repeatability of the method is expressed as a relative standard deviation (RSD). For the purpose of this method a RSD of less than 10 % may be considered acceptable, according to the requirements of Directive 98/83/EC (EC Directive, 1998) and Portuguese Legislation, Law Nº 236/1998 (PortGov, 1998) for these target compounds.

The recovery was determined by standard addition method. The washing waters spiked with an intermediate solution of the target compounds were analysed. The washing waters without fortification were also analysed under the same conditions. These studies were made for evaluate the matrix effect (washing waters) in the methods applied for the sand chemical quality. All validation studies (linearity, detection and quantification limits, precision and recovery) were studied for each parameter according with the requirements of Council Directive 98/83/EC (EC Directive, 1998) and International Procedures (ISO, 1990).

2.3. Quality of sand

In this work, the quality of sand was defined by two groups of chemical parameters: the parameters that may change during the year, mainly in summer season due to the increased number of bathers, and those parameters that may not change, but may be important for characterization of beach sands. Therefore, we verified the usefulness of sand chemical parameters to assess both contamination (pollution parameters or group II) and characterization of the sand (physicochemical parameters or group I), and evaluate their spatial and temporal changes.

The mean of three replicates of each parameter was determined. ANOVA (analysis of variance) was applied for both groups of chemical parameters in order to compare the obtained results: 1) chemical parameters *versus* sand treatment procedure; 2) chemical parameters *versus* sampling periods (individually or by group of parameters); and 3) chemical parameters *versus* beach location (individually or by group of parameters).

2.4. Beaches and sand sampling

The sand samples belong to three regions (Lisbon, Alentejo and Algarve) and a total of 15 different sand beaches were analyzed during the period 2010–2011. Table 1 shows the groups and the name of the beaches under study.

Costa da Caparica beaches occupy almost the oceanic west coast of Setubal's entire peninsula for dozens of kilometres and it is well known for its numerous beaches (38° 38′ 0″ N, 9° 13′ 30″ W). Although there is no natural division in the sandy seaside, the beaches south of the town of Costa da Caparica have different names, and sand sampling was performed in ten beaches of this group. Golfinho and Cornélia beaches are near the camping park and they are crowded during the bathing season due to the high number of campers. Contrary to other beaches of Costa da Caparica, the number of bathers is consistently high during the bathing season.

The selected beaches of Alentejo belong to Sines council which covers a vast coastal area of Alentejo, from Santo André Lagoon, in the north, to Pessegueiro Island, in the south. In this region we collected sand from three beaches: Morgavel (Mv) (37º 57' 10.78" N, 8º 51' 54.55" W), Vasco da Gama (VG) (37º 57' 10.78" N, 8º 51' 54.55" W), and Praia Grande (PG) (37º 51' 25.71" N, 8º 47' 37.44" W).

In Vilamoura, the samples were taken from two beaches, Marina beach (Mr) (37º 4' 16.52" N, 8º 6' 54.43" W) and Falésia beach (F) (37° 4' 31.00" N, 8° 7' 49.59" W). Sand samples from these fifteen beaches were grouped into two groups: (A) sand samples to study the effect of sand treatment, and (B) samples to assess the variation in the sands chemical quality during the sampling periods.

Table 1. Groups and names of beaches: (A) sand samples to study the effect of sand treatment, and (B) sand samples analyzed during the sampling periods

Portuguese Beaches	***Beaches***		
	Name (acronym)	***Group A***	***Group B***
Costa da Caparica	Praia Nova (PN)	-	PN
	Nova Praia (NP)		NP
	Cornélia (C)		C
	Golfinho (G)		G
	Mata (M)	M	M
	Riviera (Rv)	-	Rv
	Rainha (R)	R	R
	Castelo (PC)	-	PC
	Cabana do Pescador (PCP)		PCP
	Praia do Rei (Pr)		Pr
Alentejo	Morgavel (Mv)		Mv
	Vasco da Gama (VG)		VG
	Praia Grande (PG)		PG
Vilamoura	Marina (Mr)	Mr	-
	Falésia (F)	F	-

Group A includes four beaches, Vilamoura beaches (Marina Beach and Falésia Beach) and two beaches of Costa da Caparica (Rainha beach and Mata beach). The evaluation of the sand quality during the bathing season was made in all the studied beaches of Alentejo and Costa da Caparica (three and ten, respectively). Quality control of the sand during the bathing season was not conducted on the beaches of Vilamoura due to the difficulty of sampling, as the beaches are about 300 km away from Lisbon (laboratory location).

Samples were collected at a depth of about 2 cm into a plastic container using sterile gloves, and were transported to the laboratory under refrigerated conditions. The sand samples were collected in dry sand, all in the same longitudinal line of the beach, in the middle of the zone usually frequented by the beachgoers. Three samples were collected in each beach, one sample at the center of the beach (sample 2) and the other two, at three meters far from the beach boundaries (sample 1 and sample 3). These three equidistant sub-samples were mixed into one composite sample, in order to produce a 300 g sample that represented the entire length of the beach.

Every year the bathing season lasts four months, from 1st June to 30th September. Four sampling periods were planned between 2010- 2011, two out of the bathing season and two during the bathing season for a total of 49 samples at thirteen beaches in each sampling period (Table 2). All samples were processed within 48 h.

2.5. Chemical analysis

The sand treatment procedure was based on different sand washing methods: contact, stirring, sonication and boiling. For each procedure, the time, the stirring speed and the sand weight/water volume ratio were controlled. For the study of potential chemical indicators that could be used in future screenings of the beach sands' quality, two groups of parameters were chosen: physicochemical parameters (Group I) and substances regarded as pollution indicators (Group II).

The parameters of Group I include the sulphates, pH and electric conductivity. The parameters of Group II are the nitrates, nitrites, phosphates, ammonia, chemical oxygen demand (COD) and permanganate index or oxidability (the ability to be oxidized). These last parameters must be indicators of the level of organic matter in the sand because they may influence the hygienic sanitary state of the sands (ISO, 1993). The first group of parameters is only intended to characterize the washing waters while the second group aims to select the most representative parameter of the sands pollution state. The beach sand was treated with four different procedures. Two of those procedures (P1 and P2) are described in INSA Report (INSA, 2001, 2003). The other two procedures (P3 and P4) were tested by our own laboratory.

Procedure 1 (P1): 520 g of the sampled sand was mixed with 780 mL of ultra-pure water. The sand remained in contact with the water during 20 minutes, and then the solution was filtered through a 0.45 µm filter (INSA, 2001, 2003). All Group I and II measurements were corrected with respect to the weight of sand and to the volume of washing water. Due to their characteristics, pH and conductivity measurements (Group I) were not corrected.

Procedure 2 (P2): 50 g of the sampled sand was mixed with 500 mL of ultra-pure water. The solution was stirred for 30 minutes in a hot/stirring plate at 1000 rpm, and then the solution was filtered through a 0.45 µm filter (INSA, 2001, 2003).

Procedure 3 (P3): 50 g of the sampled sand was mixed with 500 mL of ultra-pure water. The solution was stirred for 30 minutes in an ultrasonic bath, and then the solution was filtered through a 0.45 µm filter.

Procedure 4 (P4): 50 g of the sampled sand was mixed with 500 mL of boiling ultra-pure water. The solution was stirred for 30 minutes in a hot/stirring plate at 120ºC. This solution was filtered through a 0.45 µm filter after reaching the ambient temperature.

Table 2. Beaches and number of samples analyzed during four sampling periods.

Beaches	***Samples**** ***(N)***	***Sampling Periods***			
		1st sampling 25th November 2010	***2nd sampling 21st March 2011***	***3rd sampling 6th July 2011***	***4th sampling 30th September 2011***
PN	3	Out of the bathing season	Out of the bathing season	Beginning of the bathing season	End of the bathing season
NP	3				
C	3				
G	3				
M	3				
Rv	3				
R	3				
PC	3				
PCP	3				
Pr	3				
Mv	3				
VG	3				
PG	3				

*Note: *For each sampling period, three sand samples were collected from each beach*

These solutions (after sand treatment procedures) were analyzed according to the requirements of the analytical methods used for water analysis. The analytical methods are the ones described in the Standard Methods for Examination of Water and Wastewater Analysis (SMEWWA) and in official procedures (Clesceri et al., 2005; International Organization for Standardization, 1993).

The sulphates were analyzed by turbidimetric method (SMEWWA 4500-SO_4 E). The nitrates, nitrites, phosphates, ammonia and chemical oxygen demand (COD) were analysed by spectrophotometric methods, such as 2,6-dimethylphenol method (SMEWWA 4500-NO_3 E), naphtylethylenediamine method (SMEWWA 4500 NO_2^- B), ascorbic acid method (SMEWWA 4500-P E), indophenol method (SMEWWA 4500 NH_3 F) and dichromate method (SMEWWA 5220-D), respectively (Clesceri et al., 2005). The permanganate index was analyzed by a titrimetric method (International Organization for Standardization, 1993). This assay was applied to the waters obtained from the sand treatment procedures, and directly to the sand samples. The direct assay was made with 10 g of sand and 100 mL of ultra-pure water. The potassium permanganate ($KMnO_4$) and potassium dichromate ($K_2Cr_2O_7$) are two oxidizing reagents, both used for quantification of organic compounds by two different methods, oxidability and COD, respectively. However, the potassium dichromate has been shown to be the most effective in the oxidation of all organic compounds, and it is the oxidizing reagent in the reference methods of the "Standard Methods of Water and Wastewater Analysis" for the determination of COD (Clesceri et al., 2005). The electric conductivity was analyzed by electrometry (SMEWWA 2510 B) (Clesceri et al., 2005). The pH was analysed by a potentiometric method (SMEWWA 4500 H) (Clesceri et al., 2005). In this study all samples were analyzed in triplicate.

2.6. Statistical analysis

Descriptive analysis for parameters was performed, using means, 95% confidence intervals for mean, median, minimum and maximum values for continuous variables. The mean values of parameters were compared between washing sand procedures, beaches and sampling period using Analysis of Variance (ANOVA) as described in section 2.3. The p value was calculated from the One-way ANOVA with three replicates for each parameter, using Excel software.

3. Results and discussion

3.1. Linearity

In order to correctly quantify the levels of the different parameters in the samples by spectrophotometry, it is necessary to study the linearity ranges. Our requirement for linearity is $R^2 \geq 0.99$, and PG values lower than the F values for the corresponding degrees of freedom (Mandel test). The coefficient of variation of the method was also determined. The obtained results are shown in Table 3.

3.2. Precision

The precision of each method (except for electric conductivity) was also evaluated by ten analyses of ultra-pure water spiked with each target compound. The relative standard deviation (RSD) varied between 0.8 % and 6.0 % (Table 4). Table 4 shows that all RSD values are below 10% and therefore the requirements of Council Directive 98/83/EC are fulfilled (EC Directive, 1998).

The precision of electric conductivity was evaluated by three analyses of each sample (washing waters). The relative standard deviation was lower than 0.5%.

3.3. Standard addition method

Ten water samples obtained after each sand treatment were analysed before and after the addition of known amounts of the target compounds under study in order to evaluate the accuracy of each method. The obtained results (Table 5) clearly showed a high accuracy of each method.

Table 3. Linear range, LOD and LOQ of the spectrophotometric methods

Parameter		*N*	*Linearity range mg L^{-1}*	*R^2*	*CV_m (%)*	*PG*	*F*	*LOD*	*LOQ*
						VT ≤ F(0,05;1; N-3)		*mg L^{-1}*	
Sulphate (SO_4)		5	5 -25	0.9950	5.2	0.40	18.5	1.4	4.7
Ammonium (NH_4)		5	0.06 - 0.24	0.9986	2.4	-1.95	18.5	0.02	0.06
Nitrite (NO_2)		11	0.03 - 0.82	1.0000	0.56	5.2	5.3	0.005	0.02
Nitrate (NO_3)	Low range	5	0.5 - 3.0	0.9972	6.0	0.53	18.5	0.25	0.84
	High range	7	4.0 - 40	0.9950	5.7	2.5	7.7	1.2	4.0
Phosphate (PO_4)		6	0.03 -0.5	1.0000	0.51	-2.3	10.1	0.005	0.02
CQO		6	3.0 - 25	0.9957	4.7	-3.00	10.1	0.56	1.9

Note: R^2=square correlation coefficients; N= number of data points; PG = test value; CVm = coefficient of variation of the method; F = value of Fisher/Snedecor (tabled value); LOD = Limit of Detection; LOQ = Limit of Quantification.

Table 4. Method repeatability in ultra-pure water spiked with each target compound

Compounds	*Fortification level (mg L^{-1})*	*%RSD (N = 10)*
Ammonium (NH_4)	0.05	3.1
COD (O_2)	10	6.0
Nitrate (NO_3)	0.5	3.5
Nitrite (NO_2)	0.009	2.1
Phosphates (PO_4)	0.064	4.0
Permanganate Index (O_2)	1.0	0.8
Sulphates (SO_4)	5.0	3.7

3.4. Sand treatment procedure

Table 6 shows the results of the group I and group II parameters for the different sand treatment procedures in the studied beach sands. Samples for which the different parameters' levels were higher than the method´s LOD were considered positive, whereas samples with concentrations lower than LOD were considered negative.

Mean concentrations of each parameter were calculated by using zero (0) for negative samples. The parameters of the Group I did not show significant differences between sand treatment procedures (p value = 0.89).

Table 5. Recovery results for each parameter in water from sand treatment procedures

Compounds	*Fortification level (mg L^{-1})*	*Recovery (%) N=10*	*RSD (%)*
Ammonium (NH_4)	0.50	90 - 108	6.8
COD (O_2)	10	90 - 109	7.8
Nitrate (NO_3)	10	91 - 108	5.7
Nitrite (NO_2)	0.050	90 - 104	4.4
Phosphates (PO_4)	0.88	88 - 112	9.0
Sulphates (SO_4)	5.0	89 - 112	7.8
Permanganate index	1.0	85 - 115	6.0

Procedure P1 appears to result in better values for electric conductivity, but these values are influenced by the higher weight of sand samples used (seven times higher). This effect was not observed in sulphates because its values were corrected for the mass of sand, and also for the volume of washing water. The results obtained show that Group I parameters are not good indicators of the sands pollution but allow the characterization of the water quality obtained from the beaches' sand treatment procedures.

The pH value of the water that resulted from all sand treatment procedures ranged between 7.8 and 9.5 with equal values of mean and median, 8.9 and 9.0, respectively. The electric conductivity ranged between 1.3 and 595 µS cm^{-1}. The mean for this parameter was 119 µS cm^{-1} but the median value was 93 µS cm^{-1}. The electric conductivity is an expression of the combined content of all inorganic substances present in a liquid and therefore can be used as an indicator of the presence of inorganic chemical contaminants. However, this parameter is not specific to individual inorganic substance. Moreover, the majority of these values are representative of the presence of low levels of inorganic substances in the sand, and for this reason parameter is not a good indicator of sand's quality. The sulphate values ranged between 0.56 mg L^{-1} and 69 mg L^{-1} with an average value of 16 mg L^{-1} and a median of 6.9 mg L^{-1}.

The analytical results of the Group II parameters showed significant differences between sand treatment procedures ($p<0.01$), and the procedure three (P3) was the best for the majority of the sands. The sonication using an ultrasonic bath was used to increase the dissolution speed by breaking intermolecular bonds. The sands washing was more effective by this procedure for extraction of the inorganic and organic substances from sands. The ammonium, nitrite and phosphate levels in the waters obtained from the sand treatment procedures were very small, and some of them were lower than the detection limit (LOD) of the methods. The nitrates concentration was higher than the above parameters, but its variability is related to the origin of the beach sand samples, and not with the sand treatment procedure.

The COD and oxidability were higher for P3 and P4 procedures. Taking into account these results, the parameters were divided in two groups: one with the inorganic parameters (ammonium, phosphate, nitrite, and nitrate) and the other with the organic parameters (COD and oxidability), and the ANOVA test was once again applied. The inorganic parameters showed no significant differences between the sand quality and the sand treatment procedures (p value = 0.11) but the organic parameters showed significant differences between the several sand treatment procedures ($p < 0.001$).

Fig. 1 shows the relationship between COD and oxidability and the sand treatment procedure (P1-P4). In environmental chemistry, the oxidability and COD test are commonly used to indirectly measure the amount of organic compounds in water. Most applications of these tests determine the amount of organic pollutants found in surface water, making these parameters a useful measurement of water quality. These tests are expressed in milligrams per liter (mg L^{-1}), which indicates the mass of oxygen consumed per liter of solution.

Table 6. Water quality from different sand treatment procedures (P1-P4) in four beaches, Mata beach (M), Rainha beach (R), Falésia beach (F) and Marina beach (Mr), n=3

	Group I			***Group II***					
	pH, 25 ºC	***Conductivity*** *µS cm^{-1}*	***Sulphates*** *SO_4, mg L^{-1}*	***Ammonium*** *NH_4, mg L^{-1}*	***Nitrite*** *NO_2, mg L^{-1}*	***Nitrate*** *NO_3, mg L^{-1}*	***Phosphate*** *PO_4, mg L^{-1}*	***Oxidability*** *O_2, mg L^{-1}*	***COD*** *O_2, mg L^{-1}*
P1-M	8.5	595	4.3	0.016	0.007	0.86	0.006	0.15	0.41
P1-R	9.4	40	3.8	0.014	0.005	0.30	0.006	0.46	1.4
P1-F	9.3	190	9.5	0.017	0.029	0.69	0.036	0.64	2.1
P1-Mr	7.8	1.3	0.56	0.004	0.007	0.19	0.029	0.77	2.4
Mean (P1)	8.8	77	4.5	0.013	0.012	0.51	0.019	0.50	1.6
P2-M	9.1	163	8.6	0.20	< LOD	3.0	< LOD	3.8	2.3
P2-R	9.0	87	6.9	0.20	< LOD	1.7	< LOD	1.8	1.0
P2-F	8.8	44	15	0.20	0.10	1.2	0.10	5.6	3.4
P2-Mr	8.8	68	< LOD	< LOD	< LOD	0.7	< LOD	4.4	2.4
Mean (P2)	8.9	90	7.9	0.13	0.03	1.6	0.03	3.9	2.3
P3-M	9.3	190	63	0.32	0.19	4.6	0.24	4.2	14
P3-R	9.5	125	69	0.35	0.22	2.9	0.23	5.9	12
P3-F	9.2	99	15	0.39	0.19	2.6	0.04	5.3	17
P3-Mr	8.8	48	42	0.47	0.18	2.3	0.24	12	24
Mean (P3)	9.2	115	47	0.38	0.20	3.1	0.19	6.8	17
P4-M	8.3	150	6.9	0.10	< LOD	4.2	< LOD	2.7	12
P4-R	8.2	104	5.9	0.16	< LOD	4.3	0.02	4.5	14
P4-F	9.5	46	5.9	0.02	0.05	0.7	0.22	3.3	14
P4-Mr	9.5	107	4.3	< LOD	0.04	1.2	0.15	3.4	11
Mean (P4)	8.9	102	5.7	0.07	0.03	2.6	0.10	3.5	13
All data by parameter									
Minimum	7.8	1.3	0.56	0.004	0.005	0.19	0.006	0.15	0.41
Maximum	9.5	595	69	0.47	0.22	4.6	0.24	12	24
Mean	8.9	119	16	0.15	0.066	2.0	0.085	3.7	8.3
Median	9.1	93	6.9	0.13	0.023	1.4	0.032	3.6	7.0

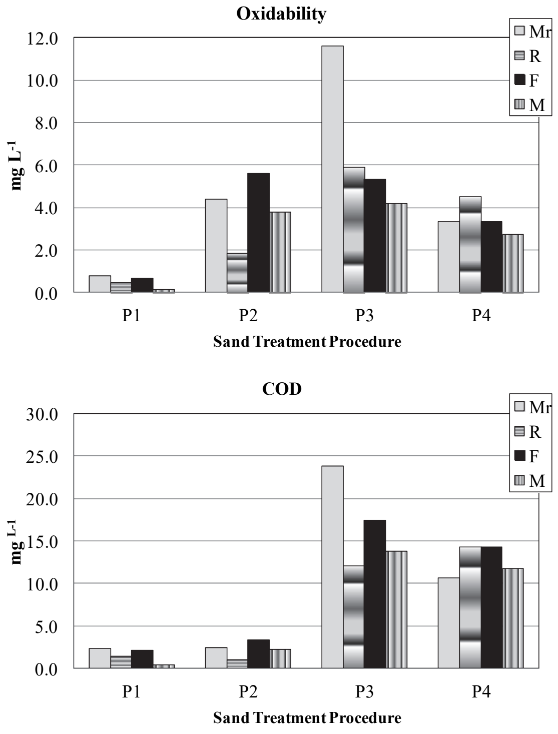

Fig. 1. COD and oxidability in waters from sand treatment procedures (P1-P4)

In this application, the beach sand treatment procedures cleaned the sands (removed the organic compounds) and the resulting washing waters were analysed. The results can be expressed in mg L^{-1} or in mg g^{-1} (according to the weight of sand used in each procedure). The different treatments applied to the sands influenced the results obtained for these parameters. The best beach sand treatments were sonication and washing with boiling water.

The solubility of one substance dissolved in another is determined by the balance of intermolecular forces between the solvent and the solute, and the entropy change that accompanies the solvation. Several factors such as temperature and pressure will alter this balance, thus changing the solubility. The solubility of a given solute in a given solvent typically depends on the temperature. Therefore, the results of P4 were expected. The organic compounds will dissolve best in a solvent that has a similar polarity such us organic solvents. However, the washing of the sands is carried out with water and for this reason other factors have been considered, such us temperature and sonication.

The solubility of the organic compounds increased with the temperature (P4) or with the sonication procedure (P3), due to the use of water in the sand treatment procedures (procedures P1-P4). Sonication and boiling water were applied to enhance the rate of dissolution. These procedures offer the possibility of achieving higher concentration of organic compounds in the waters obtained from these sand treatment procedures.

The oxidability can also be determined directly in the sand samples. The oxidability results obtained in the samples that were subject to the sand treatment procedures were compared to the oxidability results obtained directly in the sand samples (Fig. 2).

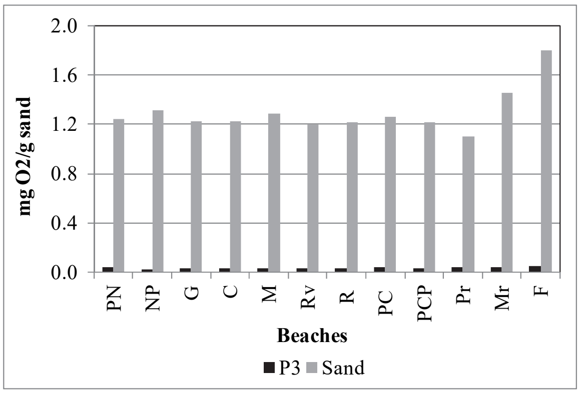

Fig. 2. Oxidability in waters from P3 procedure and determined directly in the sand

The oxidability values in the sand samples were about thirty times higher than the ones obtained with procedure P3. The efficiency of extraction was better because potassium permanganate in acidic conditions dissolves and oxidizes the sand's organic matter. Reducing species such as Fe(II) minerals will consume potassium permanganate in acidic conditions falsely indicating the presence of organics. However, this interference is common to both the direct and indirect (wash water analysis) methods and is likely more significant in the latter, as the aqueous solubility of reducing agents would increase their concentration in the wash water. Consequently, the comparative difference between direct and indirect methods may in fact be greater than what is apparent in our results.

In the following tests and due to its simplicity, it was decided to use sonication as the sand washing procedure, and the oxidability was measured after this procedure and also directly in the sand samples.

3.5. Beach sand quality

Tables 7 and 8 show the beach sand quality regarding Group I and Group II parameters during four sampling periods.

ANOVA was applied in order to compare the obtained results: each parameter *versus* sampling period, all parameters of each group *versus* sampling period, each parameter *versus* beach location, and all parameters of each group *versus* beach location.

The results showed no statistically significant differences between the beach sand quality for all parameters of both groups relating to sampling periods (Group I, p value = 0.75 and Group II, p value = 0.027), and relating to beach location (Group I, p value = 0.29 and Group II, p value = 1.0). The mean pH is 9.2 with values between 7.5 and 9.5. There are not statistically significant differences between these pH values (p value = 0.70) with respect to sampling period. However, there is a statistically significant difference between beach location ($p < 0.001$).

The conductivity varied from 52 to 382 μS cm^{-1} with a mean of 131 μS cm^{-1}, and a median of 91 μS cm^{-1}. There are not statistically significant differences between conductivity values (p value = 0.35) related to sampling period, although the conductivity values of some sand samples from Alentejo beaches (Morgavel and Praia Grande) are always higher. Therefore, there is a statistically significant difference between beach location ($p < 0.001$). The sulphates showed statistically significant differences between sampling periods ($p < 0.001$), but there are not statistically significant differences with respect to beach location (p value = 0.49). Sand samples from the first two sampling periods have slightly higher values of sulphates but within the same order of magnitude. These three parameters (pH, conductivity and sulphates) are not good indicators of the sand quality relating to sand's characterization and also to sand pollution. Therefore, they cannot be used with this purpose.

In the second group of parameters only ammonium and phosphate showed no statistically significant differences with respect to sampling periods, and their p values were 0.57 and 0.62, respectively. The ammonia ranged from 0.08 to 0.53 mg L^{-1} with similar values for the mean and median (0.20 mg L^{-1}). The phosphate ranged from 0.03 to 1.0 mg L^{-1} with similar mean and median values (0.1 mg L^{-1}). The other parameters of this group (nitrites, nitrates, oxidability and COD) showed statistically significant differences relating to the sampling periods.

Nitrite levels on the sand beach in the four sampling periods ranged from 0.01 to 0.27 mg L^{-1} with an average of 0.09 mg L^{-1}. The ANOVA detected significant variation among the different sampling periods ($p < 0.001$). The values for the first sampling period were always higher than the other sampling periods, with the exception of Praia Grande (PG) and Morgavel (Mv). Nitrate levels on the sand samples in the four sampling periods ranged from lower than LOD to 10 mg L^{-1} with an average of 3.6 mg L^{-1}, and a median of 2.6 mg L^{-1}. In some sand samples the nitrates values have doubled in the third sampling period, especially in Costa da Caparica, and in those beaches located near the Camping Park, Golfinho (G) and Cornélia (C).

This parameter was not significant in sand samples from Alentejo's beaches, and in some samples, the value was lower than the LOD (3rd and 4th sampling period in Morgavel sand). There is significant variation among the different sampling periods ($p < 0.001$) but the differences relating to beach location were not significant (p value = 0.10). There are significant differences in oxidability with respect to sampling periods ($p < 0.001$) because their values in the third and fourth sampling periods were higher, although they were always in the same order of magnitude.

Table 7. Sand beach quality regarding parameters of Group I during four sampling periods (n=3)

Sand beach		*Group I*											
		pH, 25 °C				*Conductivity* *µS cm^{-1}*				*Sulphates* *SO$_4$, mg L^{-1}*			
		1st	**2nd**	**3th**	**4th**	**1st**	**2nd**	**3th**	**4th**	**1st**	**2nd**	**3th**	**4th**
Costa da Caparica	**PN**	8.7	9.3	8.9	9.2	86	75	82	129	42	36	22	32
	NP	9.1	8.9	9.3	9.3	95	138	66	69	51	35	16	23
	G	9.1	9.3	9.3	9.3	71	119	127	62	51	40	29	31
	C	9.1	9.3	9.3	9.3	78	96	80	66	43	48	25	29
	M	9.1	8.9	9.3	9.3	85	58	75	72	46	28	37	32
	Rv	9.5	9.3	9.3	9.3	67	82	108	81	45	33	30	25
	R	9.3	8.9	9.1	9.5	119	76	360	87	45	34	37	43
	PC	9.0	9.4	9.3	9.3	57	106	268	235	50	43	41	46
	PCP	9.2	9.4	9.3	9.5	55	93	318	89	46	50	36	38
	Pr	9.1	9.4	9.2	9.5	52	61	171	119	59	32	35	37
Alentejo	**Mv**	7.5	7.6	7.6	7.6	382	366	189	273	42	41	32	42
	VG	9.1	8.8	9.1	9.1	110	87	84	98	37	43	42	49
	PG	8.5	8.3	8.2	9.0	262	100	279	266	40	40	43	41
Statistical data													
Minimum		7.5	7.6	7.6	7.6	52	58	66	62	37	28	16	23
Maximum		9.5	9.4	9.3	9.5	382	366	360	273	59	50	43	49
Mean		9.1	9.3	9.3	9.3	85	93	127	89	45	40	35	37
Median		8.9	9.0	9.0	9.2	117	112	170	127	46	39	33	36
ANOVA (sampling period)		$p = 0.70$				$p = 0.35$				$p < 0.001$			
		$p = 0.75$											
ANOVA (beach location)		$p < 0.001$				$p < 0.001$				$p = 0.49$			
		$p = 0.29$											

Table 8. Sand beach quality regarding parameters of the Group II during four sampling periods (n=3)

Sand beach		*Group II*																							
		Ammonium *NH$_4$, mg L^{-1}*				*Nitrite* *NO$_2$, mg L^{-1}*				*Nitrate* *NO$_3$, mg L^{-1}*				*Phosphate* *PO$_4$, mg L^{-1}*				*Oxidability*[1] *O$_2$, mg L^{-1}*				*COD* *O$_2$, mg L^{-1}*			
		1st	**2nd**	**3th**	**4th**	**1st**	**2nd**	**3th**	**4th**	**1st**	**2nd**	**3th**	**4th**	**1st**	**2nd**	**3th**	**4th**	**1st**	**2nd**	**3th**	**4th**	**1st**	**2nd**	**3th**	**4th**
Costa da Caparica	**PN**	0.29	0.19	0.08	0.20	0.16	0.02	0.10	0.08	2.2	6.0	1.6	2.5	0.14	0.07	0.09	0.05	4.3	3.5	3.6	3.2	26	95	155	143
	NP	0.15	0.18	0.07	0.16	0.18	0.05	0.07	0.08	4.3	4.7	2.1	1.9	0.16	0.22	0.04	0.05	2.9	3.4	4.3	3.5	13	70	150	138
	G	0.15	0.17	0.14	0.18	0.18	0.03	0.09	0.08	3.4	3.2	10	2.1	0.14	0.12	0.07	0.08	3.2	3.9	5.1	5.1	12	87	154	125
	C	0.08	0.17	0.26	0.21	0.16	0.07	0.08	0.10	3.3	4.5	5.8	2.2	0.11	0.12	0.09	0.07	3.0	3.8	5.1	5.2	10	92	160	178
	M	0.09	0.16	0.26	0.21	0.16	0.01	0.10	0.08	2.7	4.4	4.8	2.2	0.10	0.03	0.12	0.07	3.4	3.1	5.3	5.4	20	118	157	165
	Rv	0.13	0.23	0.31	0.17	0.17	0.06	0.06	0.07	3.9	3.4	6.4	2.1	0.12	0.06	0.10	0.06	3.9	3.7	5.3	4.6	36	99	160	155
	R	0.09	0.18	0.26	0.15	0.19	0.05	0.09	0.10	2.3	4.7	9.6	2.4	0.10	0.06	0.17	0.09	3.9	4.0	5.5	4.9	35	97	156	156
	PC	0.12	0.20	0.20	0.14	0.17	0.03	0.06	0.05	2.2	4.7	6.1	2.1	0.10	0.06	0.06	0.06	4.2	4.2	4.3	4.5	25	91	162	155
	PCP	0.15	0.19	0.24	0.13	0.22	0.05	0.06	0.08	4.0	5.6	7.2	2.0	0.12	0.06	0.08	0.08	3.8	4.3	4.3	5.4	19	83	158	154
	Pr	0.19	0.27	0.23	0.19	0.27	0.04	0.09	0.06	3.3	6.0	8.8	2.2	0.15	0.05	0.13	0.07	4.4	4.5	4.9	5.7	33	85	161	155
Alentejo	**Mv**	0.12	0.22	0.13	0.13	0.03	0.03	0.04	0.02	0.27	1.6	*	*	0.08	0.08	0.09	0.10	4.2	4.6	4.3	4.3	84	92	128	85
	VG	0.43	0.42	0.28	0.53	0.16	0.13	0.12	0.22	1.9	1.0	1.4	0.56	0.58	0.32	1.0	0.54	4.8	4.2	7.3	8.1	96	84	145	121
	PG	0.16	0.18	0.16	0.16	0.02	0.03	0.02	0.03	1.1	0.59	5.2	0.78	0.06	0.04	0.18	0.05	5.8	3.4	4.2	4.9	115	102	127	99
Statistical data																									
Minimum		0.08	0.16	0.07	0.13	0.02	0.01	0.02	0.02	0.27	0.59	*	*	0.06	0.03	0.04	0.05	2.9	3.1	3.6	3.2	10	70	127	85
Maximum		0.43	0.42	0.31	0.53	0.27	0.13	0.12	0.22	4.3	6.0	10	2.5	0.58	0.32	1.0	0.54	5.8	4.6	7.3	8.1	115	118	162	178
Mean		0.15	0.19	0.23	0.17	0.17	0.04	0.08	0.08	2.7	4.5	5.8	2.1	0.12	0.06	0.09	0.07	3.9	3.9	4.9	4.9	26	92	156	154
Median		0.17	0.21	0.20	0.20	0.16	0.05	0.08	0.08	2.7	3.9	5.3	1.8	0.15	0.10	0.17	0.10	4.0	3.9	4.9	5.0	40	92	152	141
ANOVA (sampling period)		$p = 0.57$				$p < 0.001$				$p < 0.001$				$p = 0.62$				$p < 0.01$				$p < 0.001$			
		$p = 0.027$																							
ANOVA (beach location)		$p < 0.001$				$p = 0.23$				$p = 0.10$				$p < 0.001$				$p = 0.080$				$p = 1.0$			
		$p = 1.0$																							

*Note: *<LOD*

However, there is not significant differences with respect to beach location (p value = 0.080). The oxidability is more representative of sand quality when it is determined directly in the sand. Fig. 3 compares both results.

For the majority of samples, the oxidability values determined directly in sand samples were two orders of magnitude higher than those from washing water (P3), and this parameter is influenced and is highest for the most crowded beaches. The oxidability values were between 0.94 and 2.4 mg L^{-1} with a mean and a median of 1.4 and 1.3 mg L^{-1}, respectively.

The values of oxidability are higher in the sand from Costa da Caparica than in the sand from Alentejo beaches (Mv, VG, PG). These results can be explained due to fact that these beaches are more crowded than the second ones, and some of them are located near camping parks. Therefore, the contamination with organic matter is higher. The COD (Table 6) varied between 10 and 178 mg L^{-1}, with an average of 108 mg L^{-1}, and there were significant differences with respect to sampling periods ($p < 0.001$). However, there were not significant differences relating to beach location (p value = 1.0).

As expected, the COD values are much higher than the oxidability values, and these values increased in the sampling periods where the number of beachgoers was higher. In the summer (third sampling period) and in the end of the bathing season (fourth sampling period) the values were higher as a result of a greater accumulation of organic matter in the sand. In some years such us 2011, when the weather was warm until the early autumn, there were many beachgoers until October, which explains the higher values in the fourth sampling period.

The COD seems to be the only parameter whose values significantly changed over the bathing season and for that reason can be representative of the number of beachgoers in a beach. Therefore, the COD proves to be a good indicator of the quality of beach sand as a parameter relating to pollution, and can be use as chemical tracer.

4. Conclusions

The described methods fulfil the criteria of linearity, sensitivity, repeatability and accuracy for routine chemical analysis of beach sands. Sonication was the most effective sand washing procedure for characterization of beach sands due to its efficiency on the extraction of adsorbed substances from the sands, mainly organic compounds.

In terms of a suitable health quality marker, none of the chemical parameters exhibited significant variation with beach location or sampling period. However, oxidability and COD values were found to be significant on the basis of sampling period ($p < 0.01$ and $p<0.001$, respectively).

The highest values of oxidability and COD in beach sand can be indicative of contamination by organic matter, with or without a human influence. These chemical parameters have remained constant outside the bathing season but they increase during this particular season. Therefore, they can be used in the future, for contamination control of beach sands. As the COD results were more representative, COD will be a better chemical indicator. The COD should be used for characterization of beach sands in other countries and those results must be addressed in order to support future decisions on environmental policy.

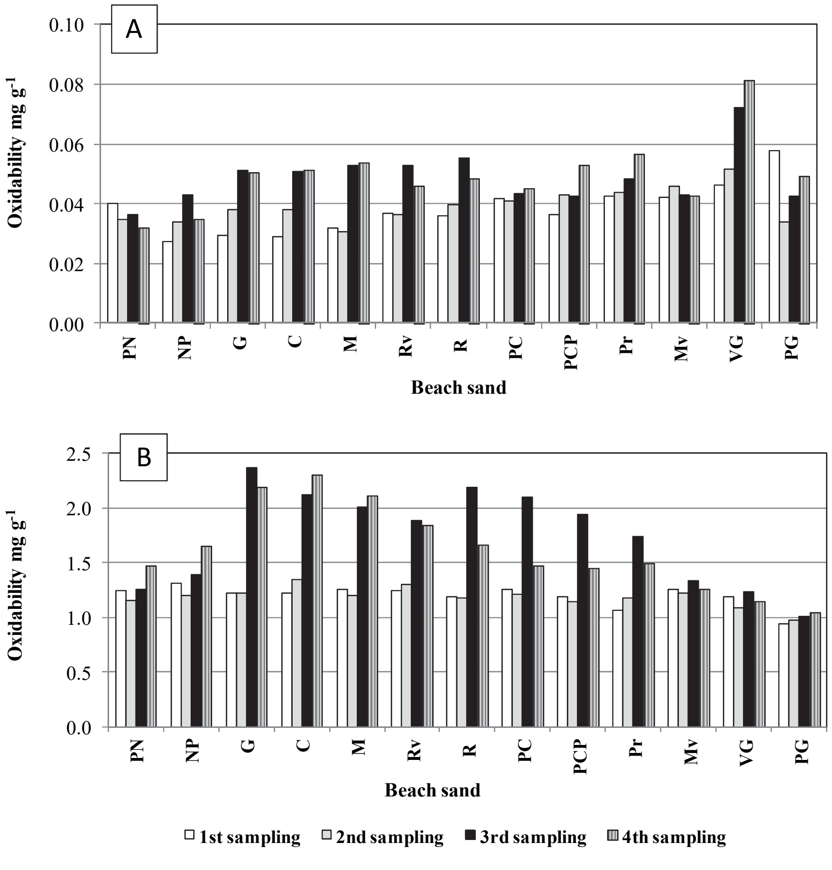

Fig. 3. Oxidability of beach sand samples determined in washing water from P3 procedure (A), and directly in the sand (B) during four sampling periods

This study should be applied in the beach sands of the other countries to a better knowledge of the abundance and distribution of these pollution chemical indicators and their behaviour during several sampling periods, mainly during bathing season.

Our results also suggest a need for a relationship between chemical measures of beach sand quality (*i.e.*, COD and oxidability) and their microbial results. As a whole, these results can contribute to a better knowledge of their negative impact on human health.

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